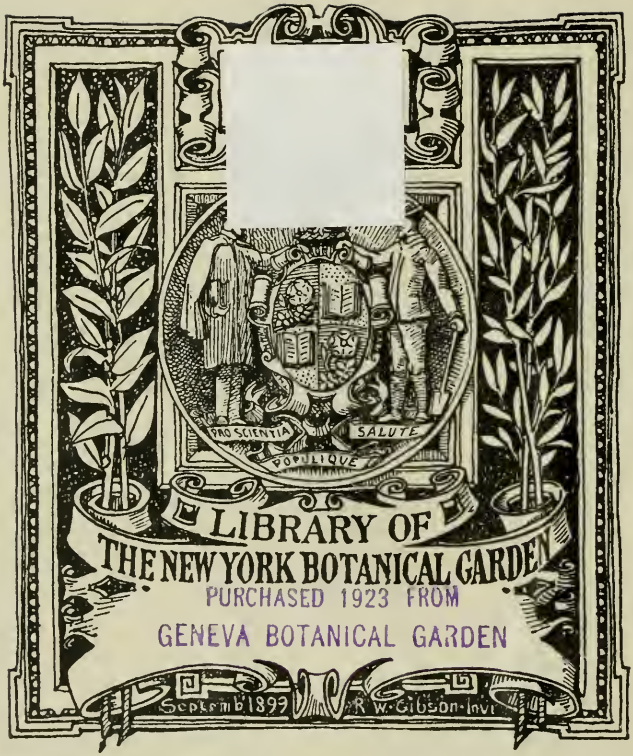
SHOUT
SHARE
IT


PRO SCIENTIA
SALUTE
POPULIQUE
LIBRARY OF
THE NEW YORK BOTANICAL GARDEN
PURCHASED 1923 FROM
GENEVA BOTANICAL GARDEN
Septemb 1899
R W. Gibson Invt

THE BOTANICAL GAZETTE

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

THE CAMBRIDGE UNIVERSITY PRESS
LONDON

THE MARUZEN-KABUSHIKI-KAISHA
TOKYO, OSAKA, KYOTO, FUKUOKA, SENDAI

THE MISSION BOOK COMPANY
SHANGHAI

THE

BOTANICAL GAZETTE

EDITOR
JOHN MERLE COULTER

VOLUME LXVIII

JULY–DECEMBER 1919

WITH THIRTY PLATES AND ONE HUNDRED FORTY-SEVEN FIGURES

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

Published
July, August, September, October, November, December, 1919

Composed and Printed By
The University of Chicago Press
Chicago, Illinois, U.S.A.

TABLE OF CONTENTS

		PAGE
Development of *Pluteus admirabilis* and *Turbaria furfuracea* (with plates I–V and eight figures)	*Leva B. Walker*	1
Development of root systems under dune conditions. Contributions from the Hull Botanical Laboratory **250** (with seventeen figures)	*W. G. Waterman*	22
Some Phyllachoras from Porto Rico (with plates VI–VIII)	*F. L. Stevens* and *Nora Dalby*	54
Apparatus for the study of photosynthesis and respiration (with one figure)	*W. J. V. Osterhout*	60
A sporangiophoric Lepidophyte from the Carboniferous (with plates IX–XI)	*Harvey Bassler*	73
Intersexes in *Plantago lanceolata* (with plates XII, XIII)	*A. B. Stout*	109
Sexuality in *Cunninghamella*	*Owen F. Burger*	134
Phytogeography of the eastern Mountain Front in Colorado. I. Physical geography and distribution of vegetation. Contributions from the Hull Botanical Laboratory **251** (with seventeen figures)	*Arthur G. Vestal*	153
On nitrification. III. The isolation and description of the nitrite ferment (with plate XIV)	*Augusto Bonazzi*	194
Polyxylic stem of *Cycas media*. Contributions from the Hull Botanical Laboratory **252** (with eleven figures)	*Ward L. Miller*	208
A parasite of the tree fern (*Cyathea*) (with plates XV, XVI)	*F. L. Stevens* and *Nora Dalby*	222
Anatomy of *Lycopodium reflexum* (with five figures)	*J. Ben Hill*	226
Origin and development of the pycnidium (with plates XVII–XXII)	*F. E. Kempton*	233
Ecology of *Tilia americana*. I. Comparative studies of the foliar transpiring power. Contributions from the Hull Botanical Laboratory **253** (with thirteen figures)	*James E. Cribbs*	262

PAGE

Repeated zoospore emergence in *Dictyuchus* (with plate XXIII and one figure) - - - - *William H. Weston* 287

Relation of nutrient solution to composition and reaction of cell sap of barley - - - - *D. R. Hoagland* 297

Chemical constituents of *Amaranthus retroflexus*. Contributions from the Hull Botanical Laboratory 254 (with eleven figures) - - - - *M. L. Woo* 313

Staminate strobilus of *Taxus canadensis*. Contributions from the Hull Botanical Laboratory 255 (with plates XXIV–XXVI and twenty-two figures) - - - - - - - - - - *A. W. Dupler* 345

Colloidal properties of bog water - *George B. Rigg* and *T. G. Thompson* 367

Vegetation of undrained depressions on the Sacramento plains (with one figure) - - - - *Francis Ramaley* 380

Relative transpiration of coniferous and broad-leaved trees in autumn and winter (with eighteen figures) - - - - - - *J. E. Weaver* and *A. Mogensen* 393

Torsion studies in twining plants (with six figures) - *H. V. Hendricks* 425

Early development of floral organs and embryonic structures of *Scrophularia marylandica* (with plates XXVII–XXIX) - - - - - - *F. M. Schertz* 441

Companion cells in bast of *Gnetum* and angiosperms (with seven figures) - - - - - - - *W. P. Thompson* 451

Secretion of amylase by plant roots (with two figures) - - - - - - - *L. Knudson* and *R. S. Smith* 460

Ray tracheid structure in second growth *Sequoia washingtoniana* (with five figures) - - - *H. C. Belyea* 467

Perithecia with an interascicular pseudoparenchyma (with plate XXX) - - - - - - - *F. L. Stevens* 474

BRIEFER ARTICLES—

A corn-pollinator (with one figure) - - - - *Merle C. Coulter* 63

Errors in double nomenclature - - - - - *J. C. Arthur* 147

Parrafin solvents in histological work - - - *Paul Weatherwax* 305

Aaron Aaronsohn (with portrait) - - - - *J. M. C.* 388

CURRENT LITERATURE - - - - - 65, 149, 232, 307, 390, 477

For titles of book reviews see index under author's name and reviews

Papers noticed in "Notes for Students" are indexed under author's name and subjects

DATES OF PUBLICATION

No. 1, July 18; No. 2, August 15; No. 3, September 16; No. 4, October 16; No. 5, November 17; No. 6, December 20.

ERRATA

Vol. LXVIII

P. 114, line 9 from bottom, for fig. 53 read fig. 52

P. 115, line 17 from top, for fig. 58 read fig. 57

P. 120, line 2 from bottom, for fig. 52 read fig. 51

Volume LXVIII Number 1

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

JULY 1919

Development of Pluteus admirabilis and Tubaria furfuracea
Leva B. Walker 1
(With Plates I–V and eight figures)

Development of Root Systems under Dune Conditions. Contributions from the Hull Botanical Laboratory 250 - - - W. G. Waterman 22
(With seventeen figures)

Some Phyllachoras from Porto Rico - - F. L. Stevens and Nora Dalby 54
(With Plates VI–VIII)

Apparatus for the Study of Photosynthesis and Respiration W. J. V. Osterhout 60
(With one figure)

Briefer Articles

A Corn-Pollinator - - - - - - - Merle C. Coulter 63
(With one figure)

Current Literature

Book Reviews - - - - - - - - - - - - 65
Life and letters of Hooker

Minor Notices - - - - - - - - - - - 65

Notes for Students - - - - - - - - - - 66

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London and Edinburgh*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Volume LXVIII Number 1

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued July 18, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, £1 12*s*. 6*d*. each; single copies, including postage, 3*s*. 6*d*. each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 18 Peking Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit. Deliveries are not guaranteed within the war zones of Europe, nor in other countries where transportation is disturbed by conditions incident to war.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

VOLUME LXVIII NUMBER 1

LIBRARY
NEW YORK
BOTANICAL
GARDEN

THE
BOTANICAL GAZETTE

JULY 1919

DEVELOPMENT OF PLUTEUS ADMIRABILIS AND TUBARIA FURFURACEA[1]

LEVA B. WALKER

(WITH PLATES I–V AND EIGHT FIGURES)

Since there is no published account of the development of any species of *Pluteus* or *Tubaria*, it seemed desirable to the writer to study representatives of these genera. Especially was this true for *Pluteus*, because in observing young stages it was difficult to determine whether the hymenophore was endogenous or exogenous in origin. The prominent cystidia, also, and the unusual structure of the trama of the gills, the filaments composing it consisting of "long cylindrical cells converging as they descend in the gills and often lying more or less crisscross at different angles of divergence," as mentioned by ATKINSON (2) in his description of *Leptonia seticeps*,[2] offered an interesting field for investigation. ATKINSON has found this structure of the trama to be characteristic of all species of *Pluteus* and *Volvaria* examined by him.

While the various species of *Pluteus* are abundant in most regions, the fruit bodies are usually formed singly or with only a

[1] Contributions from the Department of Botany, University of Nebraska, New Series, No. 27.

[2] *Leptonia seticeps* Atk. (Jour. Myc. 8:116. 1902) = *Pluteus seticeps* Atk. MSS. ATKINSON came to consider this form a true *Pluteus*. At the time he described it he placed it in *Leptonia* because of the slight attachment of the gills to the stem. His extensive studies on the structure of the *Agaricaceae* have shown that structurally it agrees in all ways with *Pluteus* and not with *Leptonia*.

AUG 7 - 1923

few closely associated. This habit makes the collection of material for developmental study very difficult. *Pluteus admirabilis* Peck occurs in troops more commonly than most other species, and its bright yellow color makes the young basidiocarps quite easily distinguishable. I began my collection of this species while in the Adirondacks on a collecting trip during July 1916. A large part of the material was collected from troops where only 3 or 4 basidiocarps of suitable age were obtainable. The largest individual collection was secured after my return to Ithaca, in the Van Samtford woods near Freeville, New York, during the latter part of August 1916.

The material for the study of *Tubaria furfuracea* Gill was collected in Cascadilla ravine, and along the banks of Fall Creek, on the Cornell University campus, during the early summer of 1916, where the plants were growing very abundantly on the leaf mold and loam in a small wood. The plants occurred in troops upon an abundant mycelium which permeated the substratum. All stages in development were to be found upon this mycelium when the fruits first began to appear.

The material for the study of both genera was fixed in chrom-acetic and Benda's fluids, cleared in cedar oil, and imbedded and sectioned in paraffin.

Pluteus admirabilis

ORIGIN OF HYMENOPHORE

No fruit body was obtained before the beginning of any differentiation had taken place in the primordium of the basidiocarp. The youngest stage secured (fig. 1) was a little over 0.5 mm. in height and already showed a differentiation into primordia of stipe, pileus, and hymenophore. The primordium of the stipe is made up of loosely interwoven, much branched filaments, uniformly about 3 μ in diameter, which become less interwoven and show many free ends near the surface, as can be seen in fig. 29, a higher magnification of the same fruit body. This tissue gradually passes into that of the primordium of the pileus, which at the base has the same structure as the primordium of the stipe, but soon opens out into a less interwoven tissue in which the filaments lie almost parallel to each other and radiate in a fanlike manner, with free

ends on the surface. These filaments are slightly larger than those of the stipe, often reaching 4 μ in diameter at their ends. This divergent growth of the hyphae making up the primordium of the pileus is similar to that described by De Bary (12, 13, 14) for *Nyctalis asterophora*, *N. parasitica*, and *Collybia dryophila*, and by Blizzard (11) for *Omphalia chrysophylla*, *Clitocybe adirondackensis*, *C. cerussata*, and *Clitopilus noveboracensis*. The primordium of the hymenophore (fig. 29) consists of only a slight modification of these outward turning filaments such as cover the pileus and stipe. They

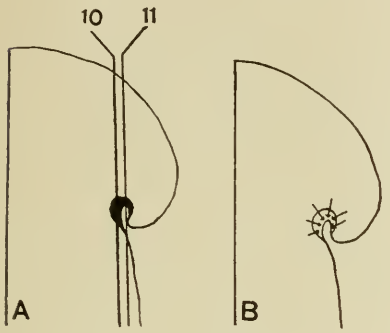


Fig. 1

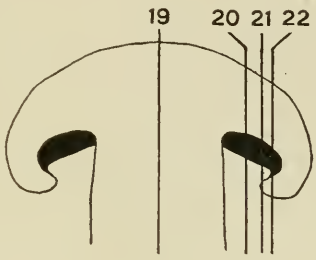


Fig. 2

Figs. 1, 2.—Fig. 1, *A*, diagram showing plane of sections shown in figs. 9–11; *B*, small arrows showing direction of growth in hymenophore of same basidiocarp; fig. 2, diagram of plane of sections shown in figs. 19–22.

are more closely packed together, smaller, and with slightly denser protoplasmic content.

Figs. 2–8 show median and tangential longitudinal sections of young basidiocarps in successive stages of development. In the fruit body shown in figs. 2 and 3, and more highly magnified in figs. 30 and 31, a definite palisade layer has been formed, and the loosely interwoven outer portion of the stem is more pronounced; otherwise the structures show no further differentiation. As growth progresses the margin of the pileus shows a strongly epinastic development, so that as the pileus enlarges the margin turns abruptly downward (figs. 4, 6), while the hymenophore still remains in the palisade condition, as shown by figs. 5 and 7. The continuation of this epinastic development causes the margin of the pileus

to roll inward so closely that the edge of the pileus becomes pressed against the loosely interwoven ends of the filaments covering the stem. Figs. 8 and 32 show a fruit in this condition with the ends of the filaments of the pileus and those of the stem intermingled. It is this condition that caused the uncertainty in the examination of young forms as to whether the hymenophore was endogenous or exogenous in origin.

The development just described reminds one of HARTIG'S (16) description of the development of *Armillaria mellea*. He found that the hymenophore developed exogenously at first, and that later by epinastic growth the margin of the pileus became incurved, and that the marginal veil developed from the interweaving of filaments growing from the margin of the pileus with similar ones growing out from the stem. This course of development, however, has been shown by BEER (10) and ATKINSON (5) not to be correct for *Armillaria mellea*. BLIZZARD (11) in his studies on exogenous forms found the pileus in several to be strongly incurved, but he did not report an interlacing of filaments from the pileus and stipe. At this stage of development the strong epinastic development ceases and the expansion of the pileus takes place more uniformly in all directions (figs. 9, 12, 15, 19, 23).

During the changes in the form of the pileus just described the general relations of hymenophore to stipe and pileus remain the same as seen in the younger stages (figs. 29, 30), but the palisade layer becomes more and more arched (fig. 32). The space between the arched hymenophore is lined with the uniform terete ends of the filaments of which the palisade layer is composed.

ORIGIN AND EARLY DEVELOPMENT OF LAMELLAE

Figs. 9–11 show low magnification of median and tangential longitudinal sections of a young fruit body in which the first trace of gill development is distinguishable. The location of these sections is shown in text fig. 1*A*, while the small arrows in text fig. 1*B* show the direction of growth in the hymenophore at this time. The gill salients arise as folds occurring in the angle between the stem and pileus where the palisade layer is first developed. The stem is narrower in the region of the developing hymenophore than below

it, so that in the tangential longitudinal section shown in fig. 10, and more highly magnified in fig. 35, the gills appear to extend as strands from pileus to stipe. The direction of the growth of the hymenophore, however, as shown in text fig. 1*B*, continues to be the same as in the level palisade condition (figs. 30, 32), so that the cells making up the fundament of the trama (fig. 35) are transverse sections of filaments extending outward at right angles to the plane of the sections. The hyphae making up the subhymenium and the fundament of the trama are smaller than in the young palisade condition.

Figs. 12–14 show a slightly older but somewhat depauperate basidiocarp in median and tangential longitudinal section. Here the young gill salients whose development is centrifugal are clearly shown. Figs. 33 and 34 show the detail of this specimen. The depauperate condition of the fruit is shown by the scanty protoplasmic content and by the enlargement of the cells to a condition characteristic of older fruits. The development having been stopped at this age, the relation of the parts would probably remain identical with those of the normally developing basidiocarps. That this would be the case is substantiated by the observation of other depauperate fruits sectioned, in which development had been arrested at all stages.

Because of the strong incurving of the margin of the pileus after the beginning of gill development, and as the expansion of the fruit body progresses along with gill development, the palisade layer constantly present toward the margin of the pileus is always on the interior (morphological under-side) of the pileus margin as seen in tangential section (figs. 15–18). In fig. 18, and better in a higher magnification (fig. 36), the palisade layer is thus shown. In figs. 19–22 a slightly older fruit body is shown, in which the hymenophore shows gills in the young salient stage on the incurved portion of the pileus. Fig. 37 is a higher magnification of the section shown in fig. 22. In this fruit body the gills are so broad that the tangential sections show the gills attached above and below, but in each case attached to the morphological under-side of the pileus, their point of origin. Text fig. 2 shows how the sections in this series were cut, and that the attachment of these gills above and below is due to the incurved form of the pileus.

The secondary gills originate at varying distances from the stem in the same manner as the primary gills, and their development progresses outward. Young secondary gills are shown in figs. 20, 22, 37, and 38. They are also well shown in a transection of the fruit body (figs. 24, 39). The primary gills during their origin and development are attached to the stem (figs. 9, 15, 30, 32), and in a transverse section of a fruit body (fig. 24), but they become free during the general expansion of the fruit body (fig. 23). In *Agaricus rodmani* ATKINSON (7) found that the gills were often attached to the stem in the early stages of development.

ORIGIN OF CYSTIDIA

During the origin of the gill salients the cystidia begin to appear (figs. 34–36). Text fig. 3 shows in outline the position of cystidia and basidia in the basidiocarp shown in fig. 36 as accurately as could be determined. As shown here, the filaments bearing both cystidia and young basidia pass out from the trama in a usually unbranched condition, the cystidia being only distinguishable from the basidia by their more scanty protoplasmic content and larger size. As the gills develop, however, the filaments leading out to the cystidia become enlarged and for some time remain little, if at all, branched, while those bearing basidia and paraphyses are smaller and much branched (figs. 38, 47, 47*a*, text fig. 4). *A* and *B* of text fig. 4 show the details of the gills at this stage as definitely as could be determined, *A* being a reconstruction of the portion shown in fig. 47*a*. A slightly older gill is shown in fig. 48 and text fig. 5, *A* showing the detail of the cystidium shown in fig. 48, and *B*, *C*, *D*, *E*, and *F* showing parts of other gills in the same series. The filaments bearing the cystidia seemingly branch somewhat during the later development and give rise to basidia and paraphyses, but even with the highest powers of the microscope it was difficult to determine positively. Cystidia

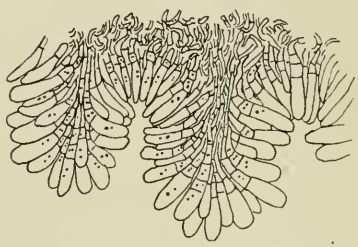


FIG. 3.—Course of filaments forming trama and leading out to young basidia and cystidia.

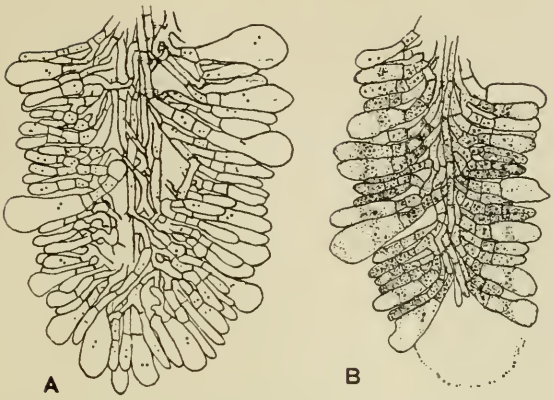


FIG. 4.—Structure of trama and origin of cystidia and basidia: *A*, reconstruction, made by aid of microscope, of photomicrograph shown in fig. 47*a*; *B*, another gill from same series outlined with camera lucida.

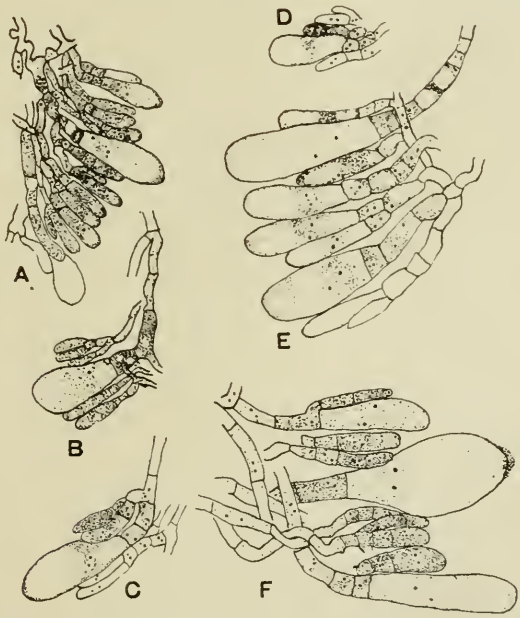


FIG. 5.—Origin of cystidia and basidia as seen in slightly older basidiocarp than that of text fig. 4: *A*, reconstruction of part of photomicrograph shown in fig. 48, made by aid of microscope; outlines of other drawings made with camera lucida.

and basidia in practically mature condition are shown in figs. 45, 46, 49, and in text fig. 6. The young basidia and cystidia, and the filaments which bear them, are constantly binucleate, as are the other cells of the young hymenophore, and in fact all parts of the young basidiocarp. The nuclei in the cystidia never fuse, and older cystidia lose their nuclei by degeneration. The cystidia are at all times much vacuolate and with scanty cytoplasm, while the basidia and paraphyses are filled with dense protoplasm. Very commonly the mature cystidium has a mucous cap (fig. 49), thus suggesting a possible excretory function, but this is not a constant characteristic.

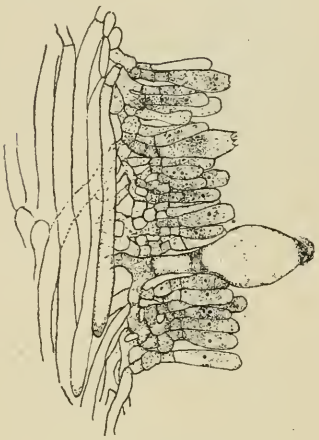


FIG. 6.—Detail of portion of gill shown in figs. 46 and 49, showing cystidia, basidia, paraphyses, and ultimate development of downward outgrowths from subhymenium (internal cystidia).

SURFACE CHARACTERS

As was noted in the description of the youngest stages of the fruit body, the outer portion of the pileus is made up of hyphae which radiate in a fanlike manner. As the development proceeds these hyphae become closely compacted (figs. 32; 33), but for some time retain this definite arrangement. The outer cells of this layer soon enlarge and appear as a uniform palisade layer, as shown in fig. 40, which is a higher magnification of the surface of the fruit body shown in fig. 19. The filaments making up the interior of the pileus branch and become much interwoven. As the pileus expands the cells forming the uniform palisade layer enlarge, giving the characteristic structure to the surface of the mature pileus (fig. 41). Structurally these cells are binucleate, and in origin seem to be homologous to the cystidia on the surface of the gills. These cells, even from youngest stages, are filled with a yellow granular content, however, and give to the pileus its characteristic color.

CHANGES TAKING PLACE IN TRAMA DURING DEVELOPMENT

As can be seen in figs. 36–38, 47, 48, and text figs. 3 and 4, the trama of the young gills is composed of slender, somewhat parallel filaments which are scarcely 2 μ in diameter at first, but soon become 2–2.5 μ in diameter, as seen in fig. 38. The trama of the gills is also very narrow and made up of relatively few filaments. As expansion of the fruit body takes place rapid changes occur in the trama. Fig. 25 is a transection of the gills of the plant shown in median section in fig. 23, where expansion is just well begun. A higher magnification of a part of these gills is shown in fig. 42. Here one observes a great multiplication of the filaments making up the trama, along with an enlargement of the individual cells. The multiplication of cells takes place largely in the region just below the hymenium, and is in the form of outgrowths of the subhymenial cells. These outgrowths extend downward and toward the center of the trama, as can be seen opposite *A* on the left of fig. 42, and in text fig. 7, which shows in a slightly diagrammatic manner the detail of this part of the section.

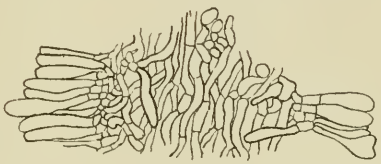


FIG. 7.—Portion of gill seen opposite *A* in fig. 42, showing beginnings of downward outgrowths from subhymenium which develop during expansion of basidiocarp.

These outgrowths continue their growth, maintaining a downward and inward direction, as is seen in a slightly older fruit body (figs. 26, 43), in which many of these filaments have reached the center of the trama. In these cells the protoplasm is much more dense in the apical end, and in many cases largely confined to this part. Figs. 27 and 44 show a still more extensive development of these filaments which have now become 4–5 μ in diameter, while figs. 28 and 45 show the gills when basidia have reached a 4-nucleate stage, but before spore formation, with the tramal cells about 7 μ in diameter. At this stage these cells are so long and thin-walled that some shrinkage takes place in all fixed material. Figs. 46 and 49 show, just below the base of the cystidium on the left, one of these filaments in good condition. This can be seen more clearly in text fig. 6. Fig. 50 shows the

trama of *Pluteus seticeps* Atk. MSS (see footnote 1), made from a freehand section which shows the typical structure of the trama of mature plants having this type of trama. The details of this can be seen more clearly in text fig. 8, which is a reconstruction from two photographs taken at slightly different foci.

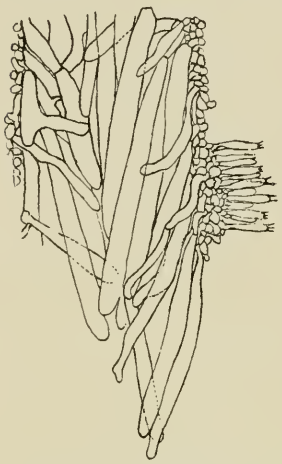


FIG. 8.—Culmination of downward outgrowths from subhymenium as seen in *Pluteus seticeps;* outlines obtained from combination of 2 photomicrographs made at slightly different focus on freehand section.

The cells composing the original trama in this species, and many other species examined, enlarge to the same size as those of the converging filaments arising in the subhymenium. This may not be true, however, of all species of *Pluteus*. In some specimens of *Pluteus longistriatus* the cells of the original trama seem to have entirely escaped the general expansion and remain slender, even in the mature pileus.

These downward growing filaments, originating as they do in the subhymenium, the same region from which the cystidia originate, and having the same general characteristics as cystidia (enlarged cells with scanty protoplasmic content), seem to represent a type of internal cystidium development. In some species of *Pluteus* a necklike constriction occurs near the apex of the external cystidium, and also on these internal outgrowths. These internal cystidia are at first binucleate, the 2 nuclei occupying a central position, as in the other cystidia, but the nuclei degenerate before the cystidia have attained the length shown in text fig. 6.

Tubaria furfuracea

EARLY DIFFERENTIATION OF PRIMORDIUM OF BASIDIOCARP

A median longitudinal section of an undifferentiated primordium of a basidiocarp is shown in figs. 51 and 78, the latter being a higher magnification of the upper portion of the former. It consists of

loosely interwoven hyphae about 2–3 μ in diameter, the external ones having firmer walls and taking the stain slightly more than those toward the interior. The fruit body illustrated in fig. 53 is smaller but shows some differentiation. There is a superficial zone made up of very loosely interwoven hyphae with firm walls. This region marks the beginning of a universal veil or blematogen layer, as this type of veil has been distinguished by ATKINSON (6). The filaments in this region show very little change in form or structure from those covering the outer portion of the primordium of the basidiocarp. Within this region is one of slightly smaller filaments with thinner walls and richer protoplasmic contents, merging into a less deeply staining region which has undergone no further differentiation. This interior zone of smaller, actively growing filaments marks the primordium of the stipe, which at this stage is somewhat conical in shape. Fig. 52 shows a somewhat older fruit body in which internal differentiation has been carried much farther. Here can be seen quite well defined the primordium of the stipe as an elongation of the conical region first differentiated, and the beginnings of the primordium of the pileus in a very slight enlargement of the apex of the stipe. The hyphae making up the densely staining region marking the primordia of stipe and pileus are smaller, about 1.5 μ in diameter, and much more closely interwoven than those of the outer portion, as can be seen in fig. 79, a higher magnification of a part of the fruit body shown in fig. 52. The blematogen and the filaments in the interior show the same characters as those of the young fruit body previously described, except that in the stipe the central filaments have come to lie somewhat parallel to each other, extending in a vertical direction. Figs. 54 and 55 show a smaller but older fruit body. The blematogen, stipe, and pileus are very clearly differentiated. As can be seen in fig. 80, a higher magnification of fig. 54, the filaments of the pileus are very compactly interwoven toward the interior, but become more and more loosely interwoven toward the margin, where they merge into the blematogen.

It is interesting to note that the stipe is usually the first region to be differentiated in several other endogenous forms, as in *Lepiota cristata* and *L. seminuda* (9), in several species of *Cortinarius* (15,

18), *Pholiota* (17), and *Hypholoma* (1). In all of these forms the differentiation of the pileus takes place in very much the same manner.

ORIGIN OF HYMENOPHORE AND ANNULAR PRELAMELLAR CHAMBER

The first trace of the origin of the hymenophore can be seen in the median longitudinal section of the basidiocarp shown in figs. 54 and 80, where the under surface of the pileus seems to take the stain more deeply. In this region the filaments are more slender, with dense protoplasmic contents, and lie more or less parallel to each other, thus marking the primordium of the hymenophore. Just below this there is a spreading of the filaments in the still undifferentiated ground tissue lying between the primordia of hymenophore and stipe, due to the rapid growth in the primordial parts. This can also be seen in the tangential longitudinal section of the same fruit body (fig. 55), which was taken midway between the stipe and the margin of the pileus. Figs. 56 and 57 are median and tangential longitudinal sections of an older basidiocarp, the tangential section representing the condition just beyond the stem. Higher magnifications of the same sections are shown in figs. 81 and 84. Here the differentiation has become much more definite. All parts of the basidiocarp are clearly distinguishable. An annular ring of definitely compacted, downward growing filaments which make up a quite clearly defined, uniform palisade layer is present, and the loosening of the tissues below the palisade layer is much more pronounced. A continuation of this development is seen in the median and tangential longitudinal sections shown in figs. 58 and 59, and more highly magnified in figs. 82 and 85. Here the palisade layer is well developed, and a more or less complete annular rift has occurred below the palisade layer, due to the continued expansion of the more actively growing parts. The filaments making up this palisade layer are scarcely 1.5 μ in diameter, and lie closely packed together and parallel to each other.

ORIGIN AND DEVELOPMENT OF GILLS

Figs. 60–63 show a fruit body when the first signs of gill development are distinguishable. The gills originate as downward growing, radial folds of an even palisade layer. These folds extend down-

ward into the annular prelamellar chamber below the palisade region. The first folding takes place near the stipe, as can be seen in the series of tangential sections shown. The details of figs. 60 and 62 are shown in figs. 83 and 86. The young gill salients thus formed by the folding of the palisade layer, along with downward growth in radial areas, develop rapidly, and as development progresses the longest gill salients are found next to the stipe and adnate to it (figs. 64–68). The folding continues outward so that near the margin we find a uniform or level palisade layer during the period of growth. Fig. 91 is a higher magnification of the hymenium shown in fig. 67. In this series, as well as in the basidiocarp illustrated in figs. 69–73, a strongly epinastic and horizontal development is observable in the pileus, and the margin of the pileus becomes strongly incurved. As can be seen by the tangential sections, the development of the gills continues to be centrifugal, and the level palisade layer is to be found on the incurving edge of the pileus. The structure of the gills at this age can be seen in figs. 87 and 88, higher magnifications of fig. 69. The trama is composed of somewhat parallel filaments that branch sparingly until the subhymenium is reached, and then branch repeatedly to give rise to the young basidia and paraphyses. Fig. 89 shows the same condition in tangential sections in a somewhat older fruit body. Figs. 74–76 show a fruit body nearing maturity. The broad attachment of the gills to the stipe, and the general triangular shape of the gills characteristic of this genus, are also shown in the median section (fig. 74), and the attachment of the gills is also clearly shown in fig. 77, a horizontal section through the pileus of a fruit body nearing maturity. As the epinastic development of the pileus continues, the margin of the pileus becomes more and more inturned. This strong incurving of the margin of the pileus results in sections similar to the ones shown in figs. 75 and 76, where the section passes through the incurved margin of the pileus as well as through the upper portion. The stalls or pigeonholes thus formed might lead one to an erroneous idea as to the origin of the gills if their development had not been traced through the earlier stages.

The secondary gills originate in the same manner as the primary gills, but at varying distances from the stipe. Sections of some of

these may be seen in figs. 76 and 92. Here some are shown that are still in the young salient condition, and two others that have started near the point at the upper portion of the pileus at which the section was cut off and developed outward. These 2 gills are distinguishable by their narrow attachment to the upper portion of the pileus. The location of the secondary gills is clearly shown in a horizontal section through the pileus (fig. 77).

The primary gills very commonly branch as they develop. The branching is frequently dichotomous, but may be of various types, as is illustrated in fig. 77. These branches seemingly arise in much the same manner as described by SAWYER (18) for *Cortinarius pholideus*.

During the expansion of the gills the hyphae making up the trama become much enlarged and separate from each other, so that a very loose open trama results. Fig. 90 shows the structure of a gill at maturity, and a comparison with fig. 89 shows the changes taking place during the expansion of the fruit body.

CHANGES TAKING PLACE IN BLEMATOGEN DURING DEVELOPMENT

As the blematogen is first differentiated it consists of loosely interwoven filaments, which take the stain quite deeply (figs. 51–55). The cells composing it soon lose their protoplasmic content, however, the individual cells becoming inflated to 4–6 μ in diameter and the blematogen showing as a very thin layer of loosely arranged, weakly staining filaments covering pileus and stem (figs. 56–73). It becomes so delicate that it is easily destroyed even with careful handling. The boundary line between the blematogen and the surface of the pileus is never clearly defined, but at all times the one merges into the other, as described by ATKINSON (4, 7) for *Agaricus rodmani*, *A. arvensis*, and *A. comtulus*, and by ALLEN (1) for *Hypholoma*. The shedding of the blematogen is somewhat like that in *Coprinus micaceus* (8) in the manner in which the cells become constricted at the cross-walls and break off, but at no time do they arrange themselves in a definite compact palisade layer as in *C. micaceus*.

During the final expansion the blematogen becomes so thin and delicate that it breaks up into scurfy particles (fig. 74), which,

together with the expansion of the cells making up the pileus, similar to that occurring in the blematogen, give the mature pileus the furfuraceous appearance characteristic of the species. The detail of the surface of the pileus at maturity is shown in fig. 93.

ORIGIN OF MARGINAL OR PARTIAL VEIL

In the early stages of the development of the pileus the blematogen is the only distinguishable layer surrounding the fundaments of the pileus and stipe, but by the time the palisade layer has developed (fig. 81) it can be seen that there are 2 distinct regions in the portion of the veil extending between pileus and stipe. The inner region is made up largely of filaments that are continuations of the more or less parallel filaments making up the margin of the pileus that extend across and merge with those of the stipe, while the outer portion is made up of the blematogen. As the annular rift becomes increasingly larger these two regions show more clearly (fig. 83). As the fruit body expands the blematogen becomes stretched into a thinner layer, but the layers can be distinctly seen in fig. 87, where the gills are quite well developed. During the expansion of the fruit body the partial veil ruptures irregularly (figs. 74, 75), and being delicate soon disappears, so that in the mature fruit body the veil is inconspicuous if seen at all.

The development of the partial veil agrees entirely with that described by SAWYER (17) for *Pholiota*, except that the veil quite commonly ruptures near the stem instead of at the margin of the pileus. ZELLER'S (19) illustrations of *Stropharia* also show a similar partial veil, but he does not describe its structure.

Summary

PLUTEUS ADMIRABILIS

1. No entirely undifferentiated primordium of a basidiocarp was obtained. The earliest stage secured showed a differentiation into primordia of stipe, pileus, and hymenophore.

2. All parts of the young basidiocarps are covered with free ends of hyphae which lie more or less parallel to each other. The primordium of the hymenophore is distinguishable by the smaller cells composing it, with denser protoplasmic contents. It develops

at the angle of junction between pileus and stipe. This soon becomes a definite level palisade layer. It is entirely exogenous in origin.

3. There is a strong epinastic development in the margin of the pileus and it becomes so strongly incurved that the filaments on its margin intermingle with those on the surface of the stipe. This occurs while the hymenophore is still in a level palisade condition.

4. The gills originate as downward growing folds which develop centrifugally, the first folding taking place at the point where the fundament of the hymenophore was first distinguishable over the angle of stipe and pileus.

5. The secondary gills originate in the same manner as the primary gills but at varying distances from the stipe. Their development is centrifugal.

6. The primary gills during their early development are attached to the stem and only become free during the final expansion of the fruit body.

7. The cystidia are distinguishable as soon as the gill salients appear. They appear as larger cells with scanty protoplasmic content, while the smaller cells of the hymenial layer are densely filled with protoplasm.

8. The cystidia are formed terminally upon filaments similar to, but usually larger than, those that bear the smaller cells of the hymenial layer. In younger stages the filaments bearing cystidia are little if at all branched, but in older fruit bodies they become more branched. The filaments bearing basidia and paraphyses branch profusely very early in their development.

9. The surface of the pileus is covered with cells that are similar to the cystidia.

10. The trama in the young gills is composed of a few slender filaments which lie more or less parallel to each other. During expansion large elongated cells developing from the subhymenium grow inward and downward, giving a very unusual appearance to the trama. These cells probably represent internal cystidia. The cells of the original trama become much enlarged also.

11. The cells in all parts of the young basidiocarps are constantly binucleate.

TUBARIA FURFURACEA

1. The primordium of the basidiocarp consists of loosely interwoven hyphae of uniform size.

2. The development of the fruit body is endogenous. The primordium of the stipe as a conical region of small deeply staining filaments is the first to be differentiated. The primordium of the pileus originates as an outgrowth of the apical end of the elongating stipe. A well defined blematogen, consisting of the undifferentiated ground tissue, surrounds the entire young fruit body.

3. The primordium of the hymenophore appears soon after the differentiation of the primordium of the pileus. At first it is only distinguishable by its deeper staining properties, but soon the filaments in the region come to lie parallel to each other and form a definite level palisade layer.

4. The development of the primordium of the hymenophore is accompanied by a stretching apart of the filaments below it to form a definite but weak annular prelamellar cavity.

5. The gills originate as radial folds in a previously uniform palisade layer. Their development is centrifugal and they very often branch toward the margin.

6. The secondary gills develop similarly but at varying distances from the stipe.

7. The surface of the pileus is never clearly defined, but at all times merges gradually into that of the blematogen. The cells of the blematogen become inflated and easily separate from each other. For this reason it is easily destroyed.

8. The marginal or partial veil is made up of 2 layers. The outer consists of blematogen, while the inner is made up largely of filaments that are continuations of those making up the margin of the pileus, and which are also attached to the stipe. The veil ruptures irregularly at maturity and is so delicate that it soon disappears.

In conclusion, I wish to express my deep obligation to the late Professor GEORGE F. ATKINSON for the use of his laboratories during the summers of 1916 and 1917, for his constant interest in my work, and for his many helpful suggestions.

UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

LITERATURE CITED

1. ALLEN, CAROLINE L., The development of some species of *Hypholoma*. Ann. Mycol. 4:387–394. *pls. 5–7*. 1906.
2. ATKINSON, GEO. F., Preliminary notes on some new species of fungi. Jour. Mycol. 8:116. 1902.
3. ———, The development of *Agaricus campestris*. BOT. GAZ. 42:241–264. *pls. 7–12*. 1906.
4. ———, The development of *Agaricus arvensis* and *A. comtulus*. Amer. Jour. Bot. 1:3–22. *pls. 1, 2*. 1914.
5. ———, The development of *Armillaria mellea*. Mycol. Centralbl. 4: 113–121. *pls. 1, 2*. 1914.
6. ———, Homology of the universal veil in *Agaricus*. Mycol. Centralbl. 5:13–19. *pls. 1–3*. 1914.
7. ———, Morphology and development of *Agaricus rodmani*. Proc. Amer. Phil. Soc. 54:309–343. *pls. 7–13*. 1915.
8. ———, Origin and development of the lamellae in *Coprinus*. BOT. GAZ. 61:89–130. *pls. 5–12*. 1916.
9. ———, The development of *Lepiota cristata* and *L. seminuda*. Mem. N.Y. Bot. Gard. 6:209–228. *pls. 21–26*. 1916.
10. BEER, R., Notes on the development of the carpophore of some Agaricaceae. Ann. Botany 25:683–689. *pl. 52*. 1911.
11. BLIZZARD, A. W., The development of some species of Agarics. Amer. Jour. Bot. 4:221–240. *pls. 6–11*. 1917.
12. DE BARY, A., Zur Kenntniss einiger Agaricinen. Bot. Zeit. 17:385–388, 393–398, 401–404. *pl. 13*. 1859.
13. ———, Vergleichende Morphologie und Biologie der Pilze, Mycetozoen, und Bacterien. 1884.
14. ———, Comparative morphology and biology of fungi, mycetozoa, and bacteria. English ed. Oxford. 1887.
15. DOUGLAS, GERTRUDE E., A study of development in the genus *Cortinarius*. Amer. Jour. Bot. 3:319–335. *pls. 8–13*. 1916.
16. HARTIG, R., Wichtige Krankheiten der Waldbäume. 12–42. *pls. 1, 2*. 1874.
17. SAWYER, W. H., JR., The development of some species of *Pholiota*. BOT. GAZ. 64:206–229. *pls. 16–20*. 1917.
18. ———, The development of *Cortinarius pholidius*. Amer. Jour. Bot. 4:520–532. *pls. 28, 29*. 1917.
19. ZELLER, S. M., The development of *Stropharia ambigua*. Mycologia 6:139–145. *pls. 124, 125*. 1914.

EXPLANATION OF PLATES I–V

The photomicrographs were made by the author as follows: figs. 1–28, 51–77 with a horizontal Zeiss camera; figs. 29–50, 78–93 with a Bausch and Lomb vertical camera and Zeiss lenses. Detailed explanations of figures may be found in the text.

PLATES I–III

Pluteus admirabilis

FIG. 1.—Median longitudinal section of young basidiocarp showing differentiation into primordia of stipe, pileus, and hymenophore; ×31.

FIGS. 2, 3.—Median and tangential longitudinal sections of slightly older basidiocarp, with well organized palisade layer; ×31.

FIGS. 4–8.—Successive stages in development of pileus; ×31.

FIGS. 9–11.—Median and tangential longitudinal sections of slightly older basidiocarp; ×31.

FIGS. 12–14.—Similar sections of depauperate fruit body in which development had been arrested when gill salients were forming; ×31.

FIGS. 15–18.—Median and 3 tangential longitudinal sections through basidiocarp just older than preceding.

FIGS. 19–22.—Position of these sections indicated in text fig. 2; ×31.

FIG. 23.—Median longitudinal section of basidiocarp nearing maturity; ×31.

FIG. 24.—Horizontal section through a basidiocarp about same age as shown in figs. 19–22, showing strong attachment of gills to stipe; ×31.

FIGS. 25–28.—Successive stages in development of gills; ×31.

FIG. 29.—Higher magnification of fig. 1, showing beginning of hymenophore; ×225.

FIGS. 30, 31.—Higher magnifications of basidiocarp shown in figs. 2 and 3, showing detail of palisade layer; ×225.

FIG. 32.—Detail of a part of fig. 8, showing interweaving of filaments of pileus and stipe, giving appearance of endogenous development; ×225.

FIGS. 33, 34.—Higher magnifications of sections shown in figs. 12 and 13; ×225.

FIG. 35.—Higher magnification of fig. 10; ×225.

FIG. 36.—Detail of section shown in fig. 18; ×225.

FIG. 37.—Detail of part of fig. 22; gill salients appear on incurved part of pileus; ×225.

FIG. 38.—Young gills from basidiocarp shown in fig. 20; ×225.

FIG. 39.—Part of hymenophore as seen on margin of pileus shown in fig. 24; ×225.

FIG. 40.—Portion of surface of basidiocarp shown in fig. 23; ×225.

FIG. 41.—Portion of surface of pileus of basidiocarp nearing maturity, showing character of surface of mature fruit, and loosening of tissues during expansion.

FIGS. 42–45.—Changes taking place in trama of gills during development.

FIG. 42.—Part of gill from fig. 25; ×225.

FIG. 43.—Part of gill from fig. 26; ×225.

FIGS. 44, 45.—Parts of gills from figs. 27 and 28; ×225.

FIG. 46.—Some of hymenium of basidiocarp shown in fig. 28; ×225.

FIGS. 47, 47*a*.—Parts of gills from basidiocarp shown in figs. 19–22, 37, 38; ×585.

FIG. 48.—Similar section to those of fig. 47 from slightly older fruit body (see text fig. 5); ×585.

FIG. 49.—Single basidium in detail; ×936.

FIG. 50.—Free-hand section of gill of *Pluteus seticeps* Atk. MSS; × 225.

PLATES IV, V

Tubaria furfuracea

FIG. 51.—Median longitudinal section of undifferentiated primordium of basidiocarp; ×33.

FIG. 52.—Median longitudinal section of early stage in differentiation of basidiocarp showing primordia of stipe, pileus, and blematogen; ×33.

FIG. 53.—Median longitudinal section of basidiocarp intermediate in development between figs. 51 and 52; ×33.

FIGS. 54, 55.—Median and tangential longitudinal sections of slightly older basidiocarp showing primordium of hymenophore as a deeper staining region on under surface of pileus; ×33.

FIGS. 56, 57.—Median and tangential longitudinal sections of basidiocarp just older than fig. 54; ×33.

FIGS. 58, 59.—Median and tangential longitudinal sections of basidiocarp with well developed palisade layer and more definite rift below; ×33.

FIGS. 60–63.—Median and tangential longitudinal sections of basidiocarp, showing first traces of gill salients; ×33.

FIGS. 64–68.—Median and tangential longitudinal sections of young basidiocarp showing centrifugal development of gills; ×33.

FIGS. 69–73.—Median and tangential longitudinal sections of older basidiocarp showing centrifugal development, attachment of gills, and breaking up of blematogen layer into flaky particles over pileus; ×33.

FIGS. 74–76.—Median and tangential longitudinal sections of basidiocarp nearing maturity; secondary gill development shown in figs. 75 and 76; ×33.

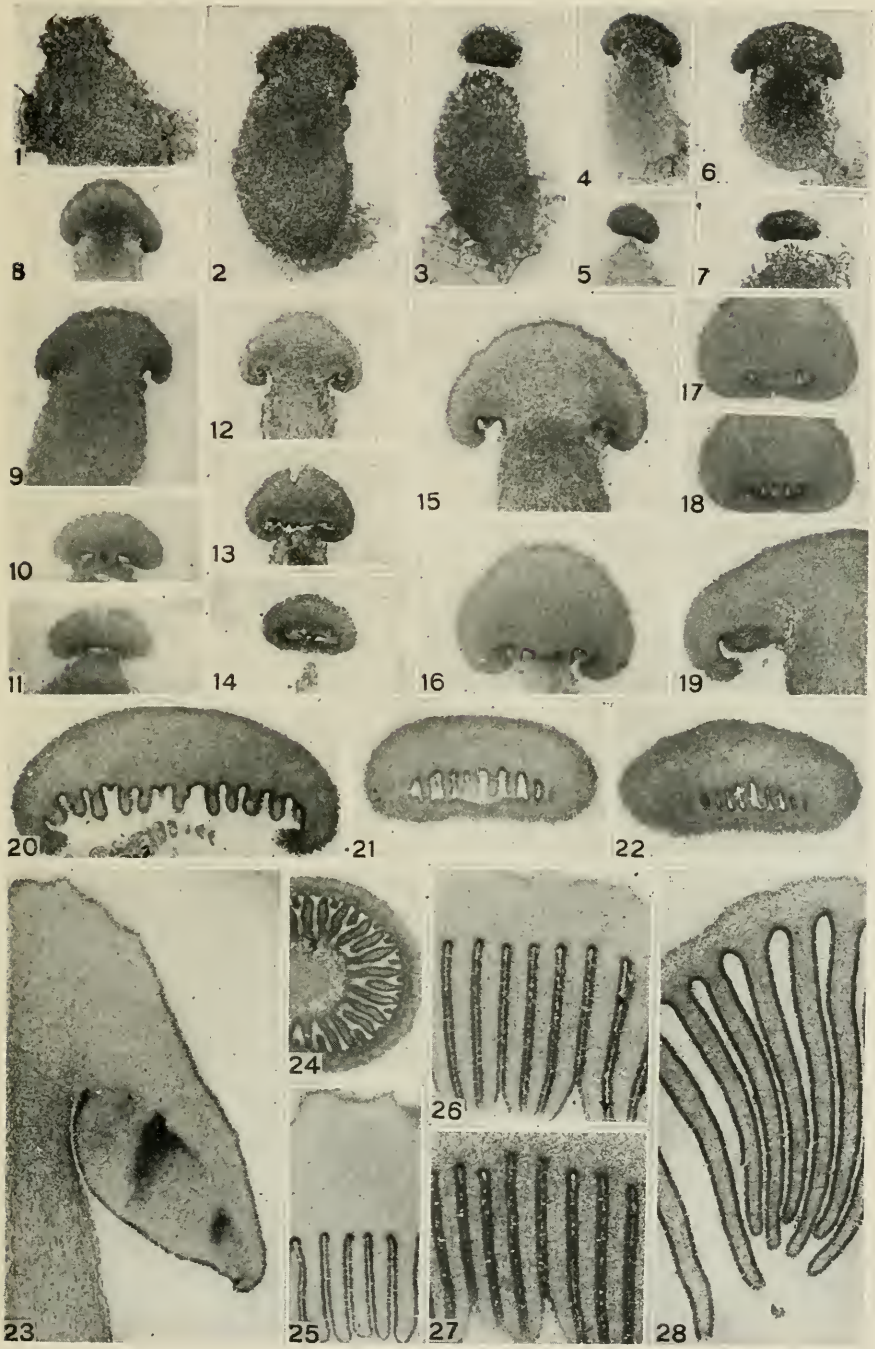
FIG. 77.—Transverse section of basidiocarp showing arrangement and branching of primary and secondary gills; ×12.

FIG. 78.—Upper part of section shown in fig. 51; ×200.

FIG. 79.—Detail of upper portion of basidiocarp shown in fig. 52; ×200.

FIG. 80.—Detail of upper portion of basidiocarp shown in fig. 54; ×200.

FIG. 81.—Upper portion of section shown in fig. 56, showing structure of hymenophore and veil; ×200.

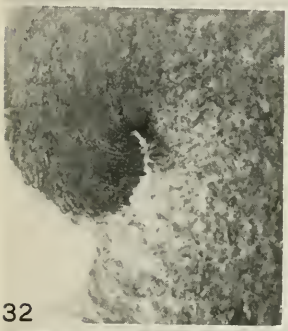


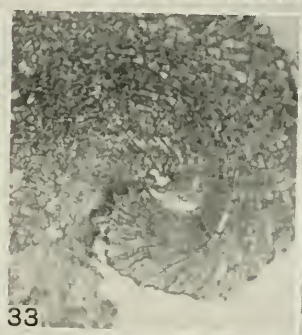
WALKER on PLUTEUS and TUBARIA

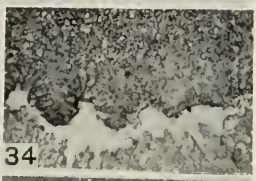
29

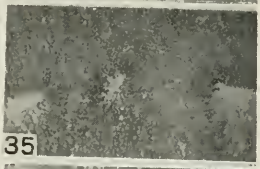
30

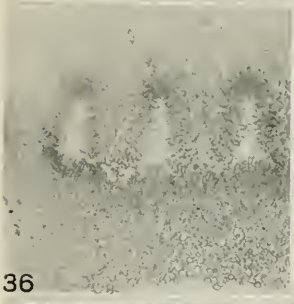
31

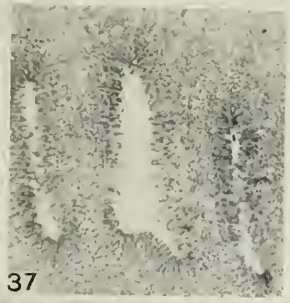

32

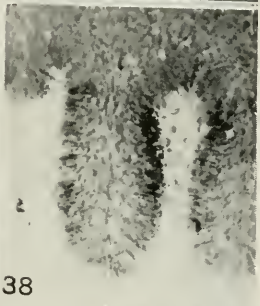

33

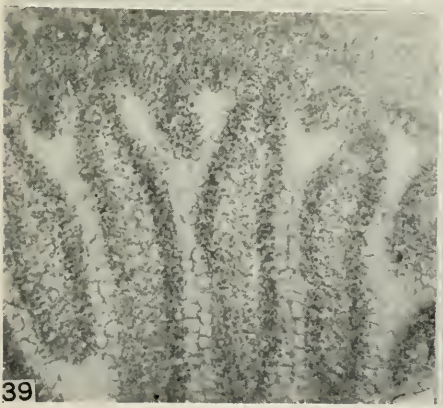

34

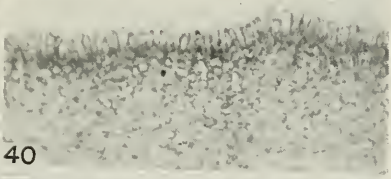

35

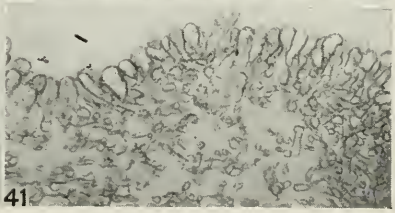

36

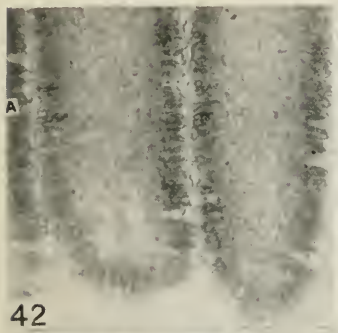

37

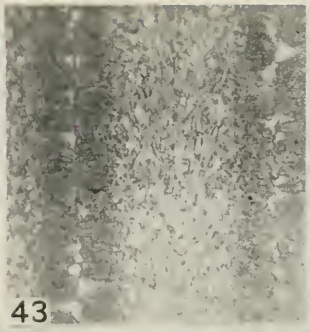

38

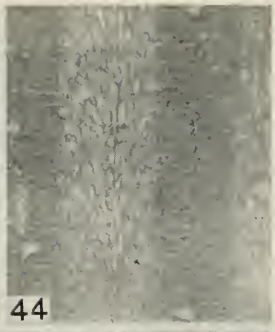

39

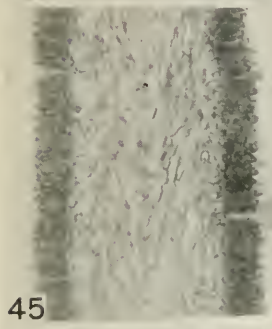

40

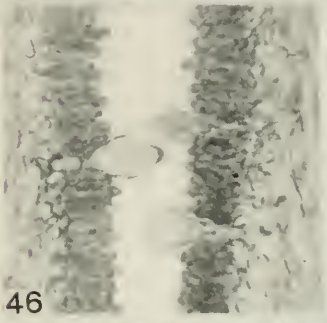

41

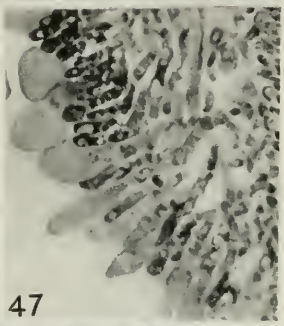


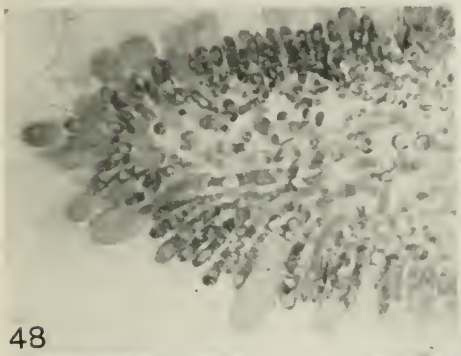


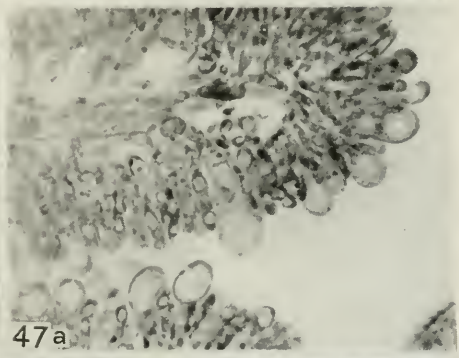


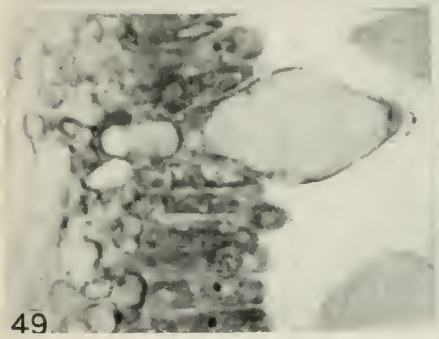


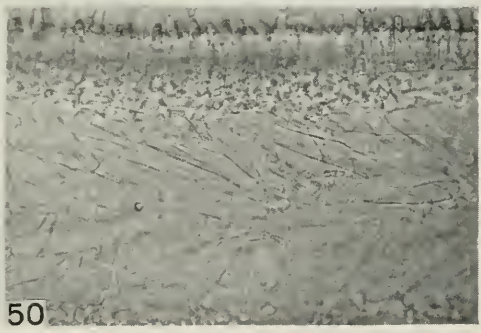




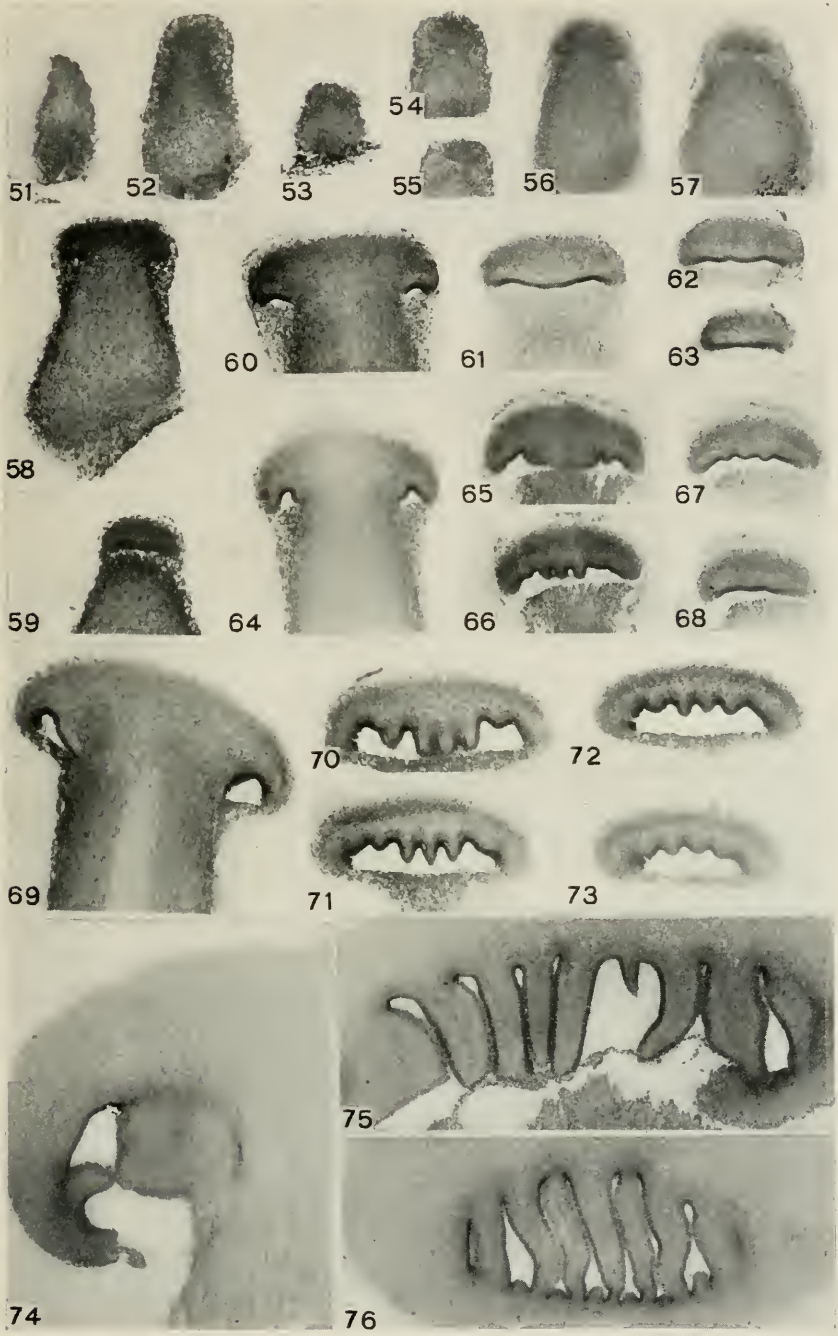




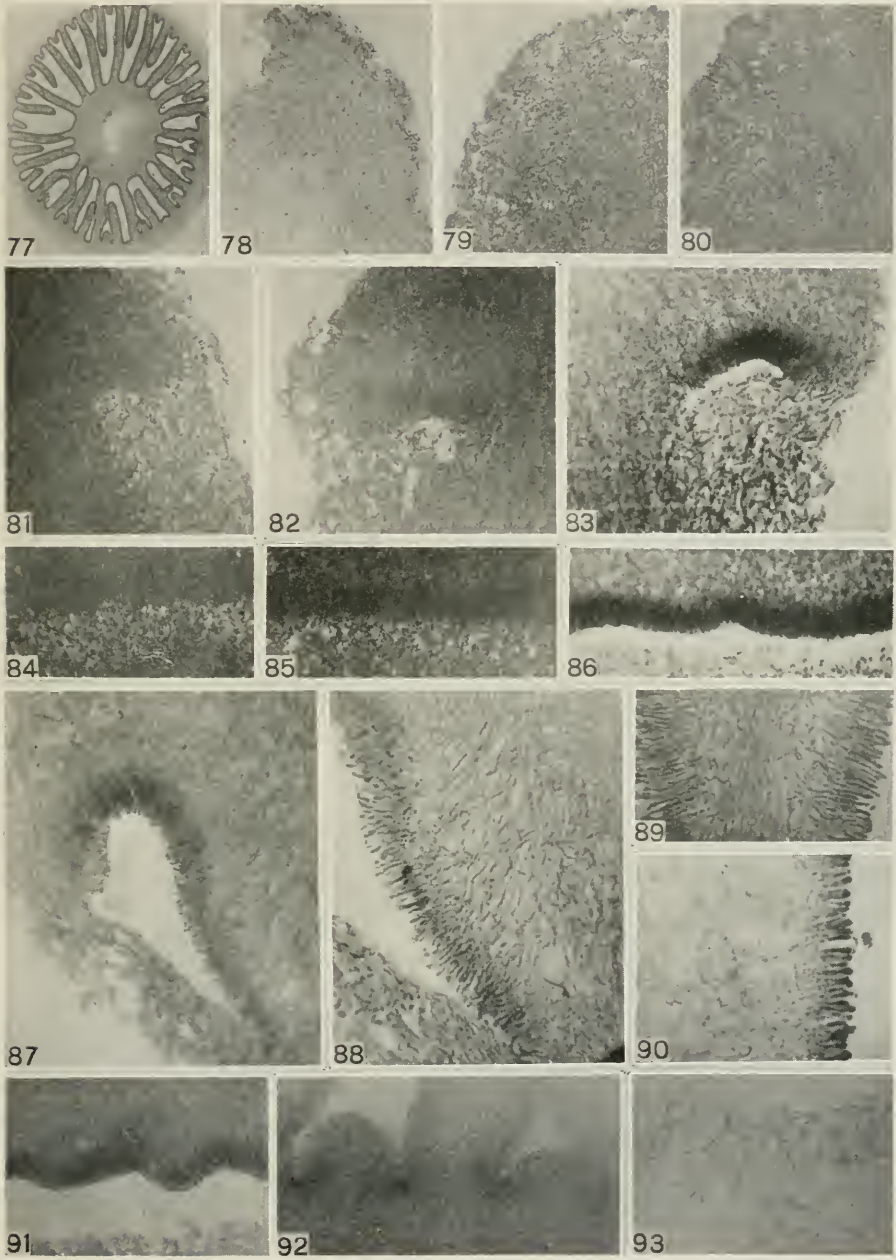




WALKER on PLUTEUS and TUBARIA



WALKER on PLUTEUS and TUBARIA



WALKER on PLUTEUS and TUBARIA

FIG. 82.—Upper portion of section shown in fig. 58; ×200.

FIG. 83.—Hymenophore at time of appearance of gill salients as seen in upper portion of section shown in fig. 60; ×200.

FIG. 84.—Part of tangential section shown in fig. 57, showing developing palisade layer; ×200.

FIG. 85.—Palisade layer as shown in fig. 59; ×200.

FIG. 86.—Young gill salients from basidiocarp illustrated in fig. 62; ×200.

FIG. 87.—Structure of gills and partial veil in basidiocarp illustrated in fig. 69; ×200.

FIG. 88.—Parallel filaments making up trama of young gills as in fig. 88; ×320.

FIG. 89.—Transverse section of young gill at about same age as preceding; ×320.

FIG. 90.—Transverse section of mature gill showing changes in trama during expansion; ×320.

FIG. 91.—Portion of hymenophore shown in fig. 67; ×200.

FIG. 92.—Part of section shown in fig. 76; ×200.

FIG. 93.—Detail of surface of mature pileus; ×320..

DEVELOPMENT OF ROOT SYSTEMS UNDER DUNE CONDITIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 250

W. G. WATERMAN

(WITH SEVENTEEN FIGURES)

The attention of the writer was first attracted to this subject by the lack of knowledge in regard to the causes of the development and consequent extension of root systems.[1] This seemed the more surprising because this knowledge is important, not only from a theoretical standpoint, but also because of its bearing on the practical activities of plant production. All of the responses of plants to soil conditions, and some of those to atmosphere, are closely connected with the condition of their root systems, but in the past both botanists and agriculturists in the main have been satisfied to interpret results by observation of the shoots only. It is evident that in all cases where the chemical and physical content of the soil is not absolutely uniform, accurate interpretation of results must take into consideration the extension and general condition of the roots.

Recognizing the practical difficulties of root observations, a locality was chosen in which the character of the soil and the scattered stand of the plants would make it possible to observe complete root systems under natural conditions. It was soon found that the distribution of nutrient material was also an easily observed and very significant factor in this locality. The region also proved to have great geological and synecological interest, and since it has been described rather fully (44) elsewhere, it will be treated only briefly here.

In the figures the root systems are arranged as nearly as possible in the natural position in a vertical plane. A 10 cm. scale was the

[1] In this study the term "development" will be applied to the process of increase in size and branching of the roots, "extension" will refer to the size of the system, and "distribution" to the relative position of the system and its parts in the soil.

standard measuring stick. When missing from any figure, the scale is the same as that of the adjoining figure.

On account of the range of the subject and the relatively small amount of work done in it, the study has been largely a survey of the ground, and the results must be regarded as indications for future work rather than as final solutions of the problems.

The work was carried on under the direction of Dr. H. C. Cowles and Dr. Wm. Crocker, and grateful acknowledgment is made to them for their advice and general assistance; as well as to Dr. G. D. Fuller, Dr. Sophia H. Eckerson, and Messrs. H. C. Sampson, E. J. Kraus, and J. T. Buchholz.

Synecology of Crystal Lake bar region

GEOGRAPHY AND GEOLOGY

The region studied is located in the northwest corner of Benzie County, Michigan, and comprises a strip of land about 1 mile wide and 5 miles long between the west end of Crystal Lake and Lake Michigan. Geologically it is probably a harbor bar formed during Algonquin time between the ends of two glacial ridges extending from Lake Michigan southeastwardly on either side of Crystal Lake. There are some indications that this strip may have a morainic core, but if so it was worked over and its top leveled off during the Algonquin high water, so that from an ecological standpoint the situation would be the same.

Soon after the recession of the post-glacial lake waters, the winds began the work of piling up dunes. Apparently they were begun much farther west on land since eroded away by the lake. The group which may be called the Point Betsie complex starts in a point on Lake Michigan at the western end of the grounds of the Congregational Summer Assembly and spreads like a fan to the north, about 2 miles in length and half a mile in width at its widest part. At its southern extremity the dunes are fixed with a very uneven contour, showing dune ridges and outlines of former blowouts, and covered by a climax forest. Approaching Point Betsie the surface is lower and the fixed dunes give place to a complex of moving sand containing residual patches of the original forest. Above Point Betsie the dunes are fixed again and end with a definite

lee slope just where the edges of the northern glacial ridge disappear under the level surface of the bar.

The glacial ridges consist of surface deposits of sand and gravel more or less water washed and stratified, but contain below at least one layer of laminated clay several feet in thickness. This layer appears on the Michigan shore bluffs and is occasionally exposed by erosion on hillsides and in ravines. On the shore end of the southern ridge is located a second small group of dunes about half a mile north of Frankfort. They are half a mile in length and one-fourth of a mile in width, and extend almost directly north from the shore, which at this point lies northwest and southeast. The group consists of small fixed dunes about 50 ft. in height, and the ridge is itself about 100 ft. above Lake Michigan. These fixed dunes have been blown out through the center in a long trough, which ends in a large, steep-sided, semicircular blowout popularly called the "Crater." For 100 yards or so from the edge of the shore bluff the sand has been blown away to or below the level of the glacial deposits, which are exposed in the bottom of the trough.

CHARACTER OF ENVIRONMENTAL FACTORS

CLIMATOLOGICAL.—So far it has been possible to obtain only incomplete and not entirely satisfactory observations, so that only a brief general statement will be given. There is nothing exceptional about the meteorological conditions of this region as to temperature, precipitation, or moisture in the air and in the soil. On account of the marked projection of Point Betsie into Lake Michigan, it is exposed both to southwesterly and northwesterly winds, which probably accounts for the large amount of moving sand around the Point. The wind also has an indirect influence on evaporation and temperature, especially in summer, as a marked difference in both is observed when a period of easterly winds is followed by a similar period of westerly winds.

SUBSTRATUM.—*Open dunes.*—On the dunes the blown sand is generally homogeneous in physical character, but a marked characteristic is the large percentage of calcium carbonate present in the form of residual grains formed by the grinding up of shells, apparently chiefly of gastropods. This calcium carbonate content

varies from 1 to 5 per cent. The dune sand is also characterized by a very unequal distribution of organic material, in the presence not only of old soil lines, but also of buried plant remains and of patches and layers distinctly different in appearance and character from the ordinary dune sand. The old soil lines, which are familiar to all who have any acquaintance with dune regions, are usually quite extensive, but generally appear only as a dark layer on the sloping sides of blowouts. Only occasionally do they occur parallel to the surface of the sand at such a depth as to affect the roots of annuals or young perennials. The upper layers of the sand, however, are generally characterized by the occurrence of layers of a dark color, usually a few millimeters in thickness and covering less than a square meter in area. The cause of the color has not been determined definitely. It is apparently carbonaceous in nature, and its source might be attributed to thoroughly decayed organic matter, and sometimes also to unusual deposits of soot from steamers passing on the lake. There is some evidence that this soot accumulates on the snow in winter and is left on the sand when the snow melts in the spring. It has been noticed also that in periods of extreme dryness a very fine powder is formed from the attrition by the wind of the exposed dead roots and stems of plants, and this is unevenly distributed by the wind over large portions of the bare sand. When fixed by a sudden shower and covered by later deposits of blown sand, very thin interbedded dark layers might be produced. Other layers and patches are marly in nature, and appear to have been deposited in beds of dried pools in former pannes. Very rarely patches of rusty color, giving an iron reaction, are found. They are only a few centimeters in length and suggest a buried nail or possibly a bit of meteoric iron as their cause.

Glacial deposits.—The upper layers of the glacial deposits are composed mainly of outwash material, sand with a few pebbles and an occasional bowlder. They are generally covered with humus, but in the crater blowout the erosion has been carried down to a gravel layer. This differs greatly from the dune sand in physical and probably in chemical constitution.

Humus.—The forested dunes as well as the glacial deposits are completely covered with a thin layer of leaf mold. This is

surprisingly uniform in depth, rarely exceeding 10 cm. in thickness. The sand below this layer has been discolored for several decimeters and its chemical content is plainly affected by materials leached down from the humus above.

Moisture content.—The moisture content of the soil varies with the location and the character of the substratum, as is shown in table I. The 7 cm. samples were usually taken in the sand just below the lower edge of the humus, as it was thought that a centimeter of depth more or less would have less significance than an indeterminable admixture of humus.

TABLE I

Locality	Depth in cms.	Wilting coefficient	Average moisture content 8 weeks July and August 1916
Open dune summit	7	0.5	2.0
" " "	25	0.5	2.5
Forested dune summit	7	1.8	2.1
" " "	25	0.3	1.3
Forested dune side	7	1.0	2.2
" " "	25	0.3	1.2
Glacial moraine	7	3.3	7.5
" "	25	2.4	5.0

ECOLOGY

CLIMAX FOREST.—The whole region, including at least part of the moving area, was originally covered by a heavy climax forest, which is still practically untouched in the southern tip and along most of the eastern edge of the dunes. The level ground on the Bar has largely been cleared, and is covered with second growth of forest trees and clearing pioneers, where not occupied by summer cottages. The climax forest is composed of beech, maple, and hemlock, with much yellow birch. The trees are tall and slender, with close stand and very little undergrowth where undisturbed. Occasional specimens of *Quercus rubra*, *Pinus Strobus*, and *P. resinosa* are found. Among the shrubs *Acer spicatum*, about at the southern limit of its range, *Viburnum acerifolium*, and *Taxus canadensis* are conspicuous. Characteristic species in the undergrowth are *Aralia nudicaulis*, *A. racemosa*, *Streptopus roseus*, *Clintonia borealis*, *Maianthemum canadense*, *Linnaea borealis*, and

Mitchella repens, with *Aspidium spinulosum*, *Adiantum pedatum*, and *Botrychium virginianum*.

Burned area.—The northern and central portions of the forested strip have been burned, in some parts repeatedly, in others not so recently. In the much burned portions the tree specimens are young and somewhat stunted. In the other portions the trees are larger and the undergrowth thicker. The species include *Betula alba*, *Prunus virginiana*, and *P. pennsylvanica*, with the more xerophytic relics of the mesophytic undergrowth, and much *Pteris* and *Equisetum*.

Border zone formation.—Where the climax forest, still untouched, extends to the shore, a zone 50–100 yards in width shows a very characteristic difference in species. The trees are *Thuja*, *Ostrya*, *Tilia*, and *Abies balsamea*, with *Celastrus scandens*. The line of demarcation is not sharp, but the climax trees, especially hemlock, mingle with the others almost to the edge of the cliffs. The characteristic border zone species are not found farther back in the climax forest.

Dune complex vegetation.—On the open dune complex there are found a number of forest patches, apparently growing in valleys between former fixed dunes whose summits have been entirely blown away. The interiors of these patches present all the characteristics of a heavy forest, and their evaporation rate is almost as low as that of the climax forest, but the vegetation is characteristic of the border zone already described, containing especially *Thuja* and *Abies*, and is marked by some trees reaching 2 ft. in diameter, but not over 30 ft. in height. The undergrowth is similar to that of the climax forest, but is especially characterized by *Viburnum acerifolium*, *Rhus toxicodendron*, and *Aralia nudicaulis*. On the edges, next to the open sand, are found *Arctostaphylos*, *Linnaea*, and *Juniperus horizontalis*. These apparently originate in the fixed area and extend out onto the sand, forming a protective covering, which frequently contains also *Juniperus communis*. Buried trees and occasional graveyards are to be found all over the moving sand area.

There is not much forest reproduction on the moving sand, and, unlike some similar regions, there are no young stands of *Pinus*

Banksiana, nor any cottonwood dunes similar to those of the Indiana-Michigan region. There are practically no panne colonies, but a few isolated oval groups, chiefly of *Thuja* and *Betula alba*, which seem to have originated from pannes, growing upward as the sand accumulates around their stems. A few other patches have reached the low conifer stage, but seem chiefly to have been invasions from the relic patches previously described. On the open sand the vegetation consists of characteristic pioneer herbs, *Ammophila* and *Calamovilfa* among grasses, with *Lathyrus maritima*, *Artemisia caudata*, *Campanula rotundifolia*, *Cirsium Pitcheri*, some *Hudsonia*, and *Zygadenus chloranthus*. There are frequent mounds protected by *Calamovilfa*, *Prunus pumila*, *Salix syrticola*, and *Cornus stolonifera*.

The growth of grasses, especially *Ammophila*, is quite extensive, and frequently approaches the character of fixed grass dunes. This is especially noticeable on the advancing lee slopes, where the complex is overwhelming the climax forest.

Literature

In view of the state of our knowledge of the general subject, the literature was reviewed rather fully, but only a brief summary of the results will be given here. The survey covered only the extension and distribution of soil roots, and the questions of absorption, structure, and effects of environmental factors were considered only as they affected extension. Owing to the range of the subject, the matter was treated from the standpoint of lines of work followed rather than that of historical development. These will be summarized and general conclusions indicated.

Intensive study of root systems.—This line has been followed mostly by German workers, and was directed chiefly toward the questions of structure and function, either in different roots of the same maturity or in the same root at different stages of its development. The leading workers along this line were von Alten (1), Freidenfeldt (16, 17), and Tschirch (43). Kroemer (24) has made the most thorough study of the "biological" significance of structure, and concludes (1) that the root is divided longitudinally into zones characterized by greater or less suberiza-

tion in the different layers of the root, and (2) that the distances of these zones from the root tip are specific. Specialized types are also reported: "contractile" (RIMBACH 30); "deciduous" and "rudimentary" (CANNON 6). Root systems are classified as "intensive" and "extensive" by BÜSGEN (4), and he regards these as hydrophytic and xerophytic, respectively.

EXTENSION.—The largest amount of agricultural work has been done by KING (23), TEN EYCK (39), SHEPHERD (36), and GOFF (18). Their conclusions were largely along practical lines and in many cases simply confirmed conclusions already reached by empirical methods. SCHULZE'S (34) work is especially to be commended for carefulness of records and character of photographs. CANNON (6, 9) has done the most comprehensive work on arid regions. His records are very complete, but his method of recording is not uniform and no scales were photographed with his plants. In several cases photographs manifestly of different enlargement were included in the same plate. On other wild herbs the best work was by WEAVER (45) on about 20 species of prairie plants. The observations were carefully made and well recorded, and the plants were photographed with scales attached. This work promises well for his "Ecology of roots" (46), soon to be published by the Carnegie Institution. This latter study was undertaken to determine the root habits of dominant and subdominant plants that were growing under a wide range of climatic and edaphic conditions; to find the root relations of the plant communities as units of vegetation; and to determine the root distribution and root competition of the individual species in their relation to other species in the vegetational group. Other aims were to determine the relation between the root habits of plants in various communities and their successional sequence; and to obtain a more definite knowledge of the indicator value and significance of various species used in classifying lands for grazing or for agriculture; as well as to aid the forester in selecting sites for afforestation or reforestation. The investigation extended over 4 years, during which time more than 1150 individual root systems of about 160 species of shrubs, grasses, and non-grassy herbs were excavated and studied on the prairies of eastern Nebraska, the chaparral of southeastern Nebraska, prairies of southeastern

Washington, plains and sand hills of Colorado, the gravel slide, half gravel slide, and forest communities of the Rocky Mountains of Colorado. Among other interesting observations on roots incidental to studies of other features, HITCHCOCK (21), PAMMEL (27), and SHERFF (37) might be mentioned. Very little work has been done on the root systems of trees. BÜSGEN (5) was not accessible to the writer. PULLING (28) describes the root systems of certain trees of the northern coniferous forest, but does not attempt much explanation of the phenomena observed.

EFFECTS OF ENVIRONMENT.—The main records of the influence of soil on the extension of root systems are incidental to other subjects, as CANNON (6, 8), HILGARD (20), HELLRIEGEL (19), and others. CRAIG (12) observed the effects of frost on the root systems of fruit trees; TOLSKY (40) made some careful experiments with oats grown at 25° and 8°, respectively; and TRANSEAU (42) observed the results of different temperatures on the growth of seedlings in bog water. CANNON (10) discusses the relation of temperature to rate of root growth. As to the depth of water table, CANNON (7) made observations on the root systems of desert plants and also on development of seedlings in relation to the water supply. CANNON and HILGARD give some information on the effects of drought, but the most extensive article by ROTMISTROV (31) was accessible only in the review in the *Experiment Station Record*. BENNETT (3) offers evidence that roots of certain land plants are not aërotropic, and CANNON (8) concludes that in mesquite and *Fouquieria* aëration within limits favors root growth and shoot development. NOYES, TROST, and YODER (26) conclude that excessive CO_2 is detrimental to normal root development, and agree with CANNON and FREE (11) as to the importance of soil aëration.

Since the work of NOBBE (25) and STOHMANN (38), HÖVELER (22), BENECKE (2), and TOTTINGHAM (41) have done the most comprehensive work on chemicals. On the whole, such work has been chiefly on the roots of seedlings, and the question at once arises whether the results would have been the same with mature root systems. BENECKE quotes PROBST to the effect that "observations on mature plants gave results opposite to those in plants less

mature, but even then the evidence seems somewhat contradictory." BENECKE concludes that, on the whole, scarcity of chemical nutrients tended to increase root length, calling this effect "hunger etiolation." HÖVELER reached similar conclusions by growing plants in alternating layers of sand and humus. SEELHORST (35), by counting the number of roots found in fertilized and unfertilized patches, decided that "in the fields investigated, plants strongly fertilized not only produced stronger roots, but also roots penetrating to lower levels." RUSCHE (32) used various salts on 8 groups of plants and concluded that the different groups responded somewhat differently, but that on the whole sulphates produced the longest and nitrates the shortest root systems. TOTTINGHAM made a thorough study of the effects of various salts on young wheat plants in water cultures by varying the proportions of the components of Knop's solution. He included observations on length and weight of roots developed, but drew no general conclusions. DACHNOWSKI (13, 14, 15) and RIGG (29) conclude that toxins in bog waters and in decaying rhizomes respectively cause stunting of roots and therefore xerophily or death of plants.

SCHREINER and REED (33) conclude that roots of healthy growing plants excrete substances deleterious to root growth, especially in plants of the same species.

Conclusions from literature

1. There has been comparatively little work done on extension of root systems as such, and the value of the results is lessened by the lack of uniformity in recording, which makes it practically impossible to compare the results of different workers. The use of vague descriptive terms in characterizing the branching of roots is also unsatisfactory.

2. Much variability of roots as a result of the action of the environment is reported, but most of this action is destructive, as the results of frost, drought, hard soil layers, etc. The experimentation with chemicals shows differences in length and weight of roots, but does not offer any definite evidence as to the causes of root extension in general or of differential extension within a single root system.

3. The elaborate studies of the structure of roots are marked in the main by great freedom of inference as to the functions of these structures. There would seem to be need of further evidence, experimental or otherwise, to give a more certain foundation for the statements as to function.

4. It seems evident that extension of root systems should be interpreted in the light of the structure and function of the roots in question. The length of a root is of little importance unless we know how much of it is functioning for the plant. Great length, also, or closeness of branching, may have very different causes and effects in different species or under different conditions, and so a very different meaning to the plant.

Root systems of dune plants

GROWTH HABITS OF ROOT SYSTEMS

METHODS.—From the consideration of the literature, it becomes evident that the extension of root systems means very little ecologically unless interpreted in the light of function and of probable causes of that extension. Studies which include simply the extension of root systems, without considering the conditions of their development and in some way evaluating the absorbing power of their different parts, omit a large part of the significant elements of the problem. For these reasons it seemed better not to attempt to study the extension of the root systems of all possible plants found on the dunes, but to confine the attention more intensively to a few species. Within the limits of the present paper it will be possible to study only the development of roots in relation to the factors of their environment. This can best be done by beginning with the germinating seed and tracing the probable course of development with the influence of the environmental factors always in mind. From this viewpoint length and weight of roots would be of less importance than the determination of a "normal" root system and the interpretation of the modifications actually found. The question of structure and function, while equally important, cannot be considered in the present paper, but must be left for future study.

General description of certain species.—*Prunus pumila.*—This was the species first investigated because, as a perennial well distributed over the Betsie complex, it showed promise of a permanent and well marked root system. On investigation it proved to have not only these features but other characteristics which admirably fitted it to serve as a basis of comparison with other species. As usually observed on the dunes, the plant has more or less of a shrub habit, with many stems caused by vegetative reproduction of parts buried by the sand. Under these conditions it frequently functions as a sand binder and forms a protected knoll or hummock. On the other hand, where the sand is being blown away, a long straggling stem will be produced, more or less prostrate and with little branching. In general the shrub type seems to fruit best, and seedlings are most abundant at the base of a shrub or around a knoll, although the fruits sometimes roll or blow some distance before being covered with sand.

The only conditions of germination seem to be burial over a winter in 1 or 2 inches of sand. In the laboratory the seeds germinated readily in a sterile moist chamber on filter paper, after the stony pit and the seed coat had been removed.

After 3 months' growth on the dunes the root system shows the general type illustrated in fig. 1, while a 3-year-old seedling is shown in fig. 2. The root system, however, does not usually develop along these lines. The more usual form is extremely asymmetric and develops very unevenly, as indicated in fig. 3. All the seedlings show wide individual diversity of form, and these specimens are not at all exceptional in their eccentricity. An examination of these asymmetric forms shows that their irregularities are connected with the distribution of more or less decayed plant parts under the sand. A typical illustration of this is shown in fig. 4, where horizontal laterals are shown passing through and exploiting bits of stems, branches, etc. When a seedling grows in the vicinity of a dark layer already described, its laterals are generally found in these layers, and they are more branched and longer than those in ordinary dune sand; in fact, a layer of this sort will generally be found to contain many rootlets of various species of plants, while the sand on either side is almost completely free from them. An interesting

case was found in the sand at Miller, Indiana, shown in fig. 5. A 3-year-old specimen (*b*) grew within the field of such a layer, and the effect is seen in its well developed horizontal laterals. The carbonaceous layer in question was absent in the neighborhood of

FIG. 1

FIG. 2

FIGS. 1, 2.—*Prunus pumila:* fig. 1, 3 months old; fig. 2, 3 years old

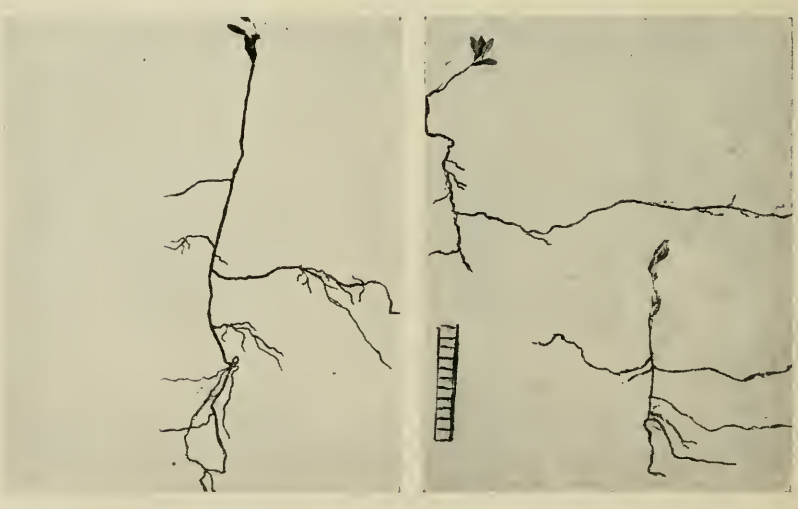


FIG. 3.—*Prunus pumila,* showing asymmetric habit

the 2-year-old specimen *a*, barely 2 ft. away, and the absence of prominent laterals is very marked. Cases of extreme elongation were occasionally observed, as in fig. 6. In the specimen figured, the root came in contact with a decaying grass rhizome and turned back at a sharp angle, following the rhizome for about 60 cm. In a similar case the root did not make such a sharp angle, but followed the rhizome for an even greater distance. It should be noted in

FIG. 4

FIG. 5

FIGS. 4, 5.—*Prunus pumila:* fig. 4, showing relation of roots to buried plant parts; fig. 5*a*, 2 years old, which grew in normal dune sand; *b*, same, 3 years old, which grew in sand with interbedded black layers.

both cases that the size of the shoot was not at all in proportion to the length of the root.

In these cases the relation of the root to the organic matter is not clear. Generally there is little penetration of the tissue of the decaying organ. There are occasionally short laterals clasping the foreign body, frequently passing under a sheathing leaf or a disorganized epidermis, and in cases of extreme decay adjusting themselves to the easy passages formed by the disintegration of the middle lamella of the cell walls. There are no indications of haustoria or of actual penetration of cell walls. In black layers and

patches in the sand there are cases of extreme development of small rootlets with close branching.

The development of the shoot is more or less connected with the presence of these plant parts. Seedlings whose roots do not come in contact with such organic matter have few and small leaves, and in general it would seem that the securing of food from such organic matter is essential to the development and maturity of the plant. Most of the plants which have reached the shrub stage are found to have stems which have apparently come up through superposed masses of sand, and while it has not been possible to demonstrate the presence of organic matter at the base of such shrub systems, such presence is very strongly connoted by the appearance of the plant and the evidence from the smaller specimens excavated. The condition of the shoots with very long roots will be considered later.

FIG. 6.—*Prunus pumila* with long lateral developed in contact with dead *Ammophila* rhizome.

In the sphere of inhibiting factors very little evidence was observed. On account of the scattered stand the roots seldom come into contact with roots of other plants, but occasionally they were found exploiting bits of buried plant material in company with and unaffected by roots of (*a*) other species. In one case, however (fig. 7), a *P. pumila* seedling had sent its roots down almost into a thick mat of willow roots (*b*). Here there seems to have been a very definite dwarfing of the root system, either from the presence of injurious excretions or because of the removal of water or nutrient material by the willow roots.

The presence or absence of the water table seemed to have no directive effect on the *P. pumila* roots; in fact several seedlings

were found growing in the water on the edge of a pool, with no apparent effect on the roots. Observations near Miller, Indiana, on *P. pumila* and *Populus deltoides*, seemed at first to show such effects (fig. 8). The seedlings were growing in very wet sand with the water table only 8–10 cm. below the surface. On inspection the water table zone proved to be very mucky and foul, and the effects on the taproots seemed to be a rotting due to the action of micro-organisms, with a proportionately increased development of laterals.

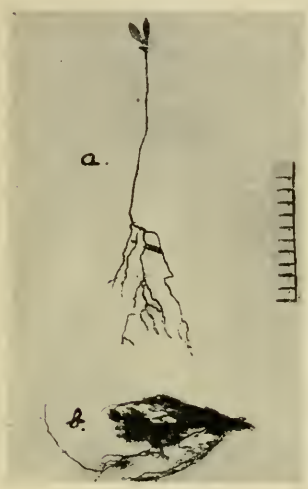


FIG. 7

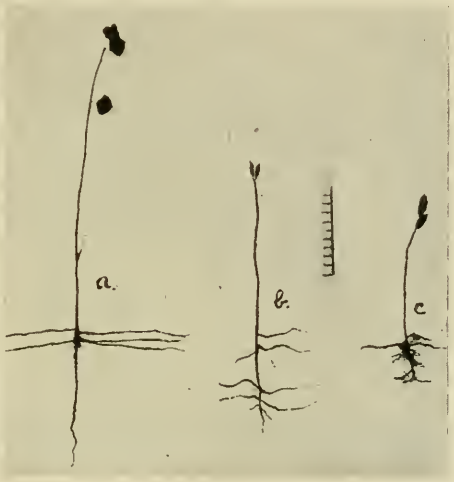


FIG. 8

FIGS. 7, 8.—*Prunus pumila:* fig. 1*a*, with stunted root system; *b*, horizontal layer of matted willow roots in approximately natural position with relation to *P. pumila* roots; fig. 8, root systems showing effects of high water table, Miller, Indiana: *a*, *Populus deltoides; b* and *c*, *Prunus pumila*.

Ammophila arenaria.—While this is the typical plant of the open dunes, its seedlings are difficult to find, and its reproduction is mostly vegetative through rhizomes. The spikes are thoroughly exploited by insects, and when gathered late in the season very few seeds will be found untouched. For this reason the seedlings are very scanty, except in dense colonies where mature spikes have been buried by fresh sand, and even then there are seldom over 6 seedlings from a whole spike. In the mature plant, as is well known, there is a long slender rhizome producing usually 2 roots

and a bud at each node. The bud frequently does not develop, but the roots range widely under normal conditions through fresh blown sand, often reaching a length of several meters. They are thickly set with short laterals which bear abundant root hairs. At times the tips of these roots are much enlarged for about 10 cm., gradually tapering in both directions and without branches. All the roots are extremely wiry and tenacious, and on account of their fineness and length cannot well be shown in a figure.

It has generally been recognized (WESTGATE 47) that *Ammophila* ordinarily requires annual supplies of fresh sand for its best development. Field observations in this region would indicate also that it does not thrive in sand possessing an appreciable amount of humus, and that its colonies do not extend across the border into such a region (sand containing humus). In at least one case a colony seems to be dying out in the presence of a competing colony of *Calamovilfa*, which has not been studied closely, but which seems to be more humus tolerant than *Ammophila*. Excavation shows that the roots of mature plants of *Ammophila* exhibit little response to organic material, following black layers only slightly and frequently passing obliquely through them. A study of the seedlings would seem to indicate that the extension of their roots may to some extent be inhibited by the presence of decaying plant parts. In fig. 9, *a* grew in pure sand, but in *b* all the roots came directly into contact with buried *Ammophila* leaves and stems, and in *c* the upper roots developed in pure sand and the lower in plant remains. It will be noted that in *b* and *c* the roots do not extend beyond the

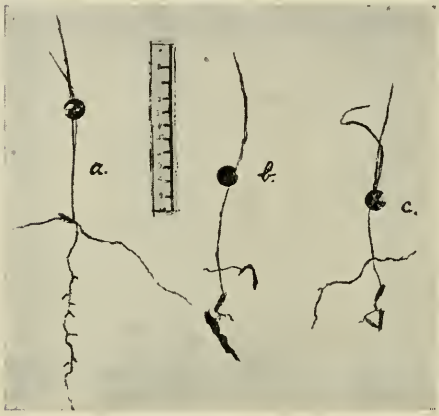


FIG. 9.—Seedlings of *Ammophila arenaria*, 2–3 months old: *a*, in homogeneous dune sand; *b*, all roots in contact with buried plant parts; *c*, upper roots in sand, lower in contact with buried plant parts.

decaying plant parts. Great care was taken in excavating these specimens, which are simply typical of a number of cases found, and there can be no question that in every case the roots stopped in the decaying plant parts as figured.

Artemisia caudata.—This species germinates freely on the open dunes, and also in the edge of the forested sections. It is described by GRAY as not perennial, but the character of the mature specimens found on the dunes would indicate that there at least it has a perennial habit. Two series of plants are shown, one grown in pure sand (fig. 10), the other in sand containing some admixture

FIG. 10

FIG. 11

FIGS. 10, 11.—*Artemisia caudata:* fig. 10, which grew in pure dune sand, showing stumps only of long laterals; fig. 11, of apparently same ages as in fig. 10, but in sand containing some humus; laterals very short.

of humus (fig. 11). In the former may be observed the stubs of the characteristic laterals, which in mature specimens extend 20–30 ft. No attempt has been made to show in the figure the full extent of these laterals, but rather their relation to each other and to the taproot. In pure sand this species shows some of the asymmetry of *Prunus pumila*, but the causes are not so evident. In some specimens very long laterals develop almost on the surface of the sand, and are so shallow that they are often exposed and killed by the blowing away of the upper dry sand layer. In other specimens it is one or more of the deep seated laterals which shows

extreme elongation. The superficial laterals show marked parallelism with the upper surface of the sand, even in plants growing on slopes; where the laterals join the main root at different angles, they are acute on the upper side and correspondingly obtuse on the lower. The position of these laterals seems to be on the relatively constant plane separating the upper layer of dry sand from the moist sand which constitutes the remainder of the substratum. In general the system is characterized by extreme shortness of taproot, but occasionally the taproot becomes very prominent.

FIG. 12

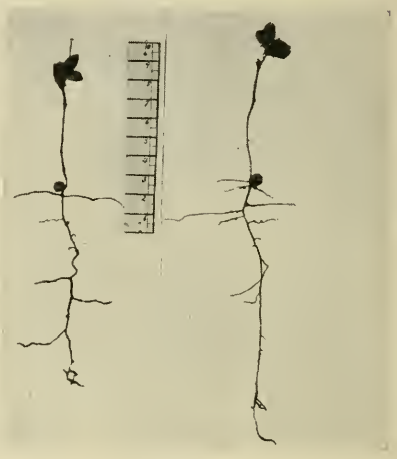


FIG. 13

FIGS. 12, 13.—Fig. 12, *Campanula rotundifolia: a*, normal root habit; *b*, young plants on exposed edge of horizontal marly layer; fig. 13, seedlings of *Lathyrus maritima*, showing remains of seed and root tubercles.

Artemisia resembles *Ammophila* in the reduction in length of roots in the presence of organic matter or humous layers, and its roots are not attracted by decaying plant parts. It will be noted in fig. 11 that the laterals of this series, which grew in sand mixed with humus, are finer, more branched, and with none of the very long laterals of the pure sand specimens. The fact that the roots are not attracted by decaying plant parts was distinctly shown in one case where a mature plant with laterals up to 6 ft. in length was growing near a decaying log. One lateral passed a few inches below the log in a zone of leaching, bent first away, then toward the log,

then away again, and showed in general no effect attributable to the presence of the log. A marked attraction would have been expected if the plant had been *P. pumila*.

Cirsium Pitcheri.—This is apparently ecologically similar to *Artemisia caudata* as to locality and general conditions of growth, but it differs in having a strongly developed taproot with very few and inconspicuous laterals. Occasionally, for no apparent reason, a single lateral becomes prominent, as in *Artemisia*.

Campanula rotundifolia.—This species is not uniformly distributed, but apparently germinates freely in restricted localities on the open dunes, as well as in open places of a humus bearing substratum. Its marked characteristic on the dunes is the long taproot with U-shaped insertion of laterals (fig. 12*a*). In one case where a buried marly layer was exposed on a slope a row of seedlings was observed growing along the exposed edge of the layer, and the roots were found to have grown horizontally inward through the marly layer (fig. 12*b*). They have not been observed in contact with the dark layers, so their reaction to them cannot be stated.

Lithospermum Gmelini.—This is a fairly well distributed plant on the open dunes, and is characterized by a very long, thick, black taproot, with almost no laterals. A study of the structure might reveal some interesting features as to the absorbing power of this root.

Lathyrus maritima.—This species is frequently the companion of *Ammophila*, but it differs in root development in some important respects. It germinates freely on the open dune, producing numerous seedlings. No marked seedlings have been followed over winter, but from the scarcity of 2-year-old plants they do not seem to survive well, either on account of sand movement or possibly because of lack of nutrition. The mature plant develops a wide ranging rhizome, deeply placed and difficult to excavate unless exposed in a blowout. The roots are scattered and usually not over 10–20 cm. in length. Root tubercles are early developed in dune sand (fig. 13), but less in some localities than in others, possibly on account of unequal distribution of infecting organisms. When present the tubercles occur only near the surface of the sand,

they do not occur in a humous layer, and in humus the roots are longer than in sand.

Several species found more or less frequently on the open sand, but especially along the borders of forested patches being blown out, have in general similar characteristics, and will be described together. These are *Thuja occidentalis*, *Cornus stolonifera*, *Arctostaphylos Uva-ursi*, *Vitis* spp., *Betula alba*, *Tilia americana*, and *Juniperus horizontalis*. These rarely germinate on the open sand, although *Thuja* and *Betula alba* are occasionally found germinating on the edges of blown out patches, and regularly germinate, when present, on the floor of these same patches. A study of their root systems shows that the roots regularly follow and exploit the carbonaceous layers and old soil lines, and the plants remain stunted unless their roots find such plant remains.

Populus deltoides.—This species is very rare in this region, and its ecological equivalent, *P. balsamifera*, is found sparingly along the shore of Lake Michigan, more frequently on Crystal Lake beach, and in the burned part of the forested dune area.

Salix spp.—The willows were not included in this study, although their roots were frequently met, and certain points may be noted. They showed a positive and vigorous hydrotropism, which was observable in the neighborhood of pannes and also of Lake Michigan. Several times roots were found descending with the slope of the surface of the sand until they finally entered the water table and passed under the pool in the panne. They also show in marked degree the ability to form a small bunch of closely branched rootlets in small dark patches in the sand.

GENERAL OBSERVATIONS

Root systems in glacial gravel layers.—In the blown out portions of the Crater group gravelly layers of the glacial substratum had been laid bare and bore a scattered vegetation similar to that of the dunes. There was some excavation of *Prunus pumila* and of *Artemisia* in this locality, and indications seemed to point to a closer and more regular branching. There were of course no buried plant parts, so that asymmetric development would not be expected (fig. 14).

Mycorrhiza.—The question of symbiotic fungi suggested itself early in the investigations, but there was no opportunity for a detailed study. Slight examination of a few species was made, but did not show evidence of the presence of such fungi.

Enlarged root tips.—A frequently observed feature of many of the dune species was the marked enlargement of some root tips. The tip rapidly increased to 3 or 4 times its normal diameter and then gradually tapered away again, the extreme length of the enlargement being 20–30 times its greatest diameter. This enlargement seemed to be confined to the central tip of the larger branches only,

FIG. 14.—*Prunus pumila* plants in sandy morainic substratum

and especially in those which showed a marked and rapid extension into new territory, for which reason the name "pioneer" or invading root tip has suggested itself for this type. It was found under apparently normal conditions both in pure sand and in humus, but in the sand it seemed to be increased in some species by the chemical solutions used, and even by distilled water. The largest tips found were in the case of *Juncus balticus,* where in shape and size they resembled large angleworms. It was not possible to make a microscopic examination of fresh material, but sections of tips preserved in 4 per cent formaldehyde showed no signs of symbiotic fungi or animal forms. The enlargement seemed to be due to increase in size and number of the parenchyma cells.

In several cases there were indications that taproots and laterals gave different reactions to the same stimuli. In these species taproots were never deflected by horizontal black layers or plant parts, while laterals were apparently free to move in any direction. Typical species showing this habit were *Artemisia* and *Cirsium Pitcheri*, while in *Prunus pumila* and notably in *Campanula* the taproots, if such they could be called, showed marked variability in a horizontal direction. Whether this difference was structural

FIG. 15

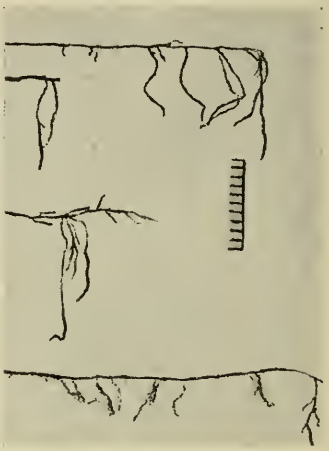


FIG. 16

FIGS. 15, 16.—Fig. 15, *P. pumila* root system after 2 months' irrigation with Knop's solution; round black spot represents location of center of diffusion and dotted circle apparent limits of influence; practically all new roots found within the circle; fig. 16, *Thuja occidentalis:* new root tips grown under irrigation with Knop's solution.

or due to a difference in quantity or quality of the stimulus was not evident.

EXPERIMENTATION

A consideration of these observations would suggest several possible factors as causes for the observed characteristics of root systems of dune plants. Chief among these would be the distribution of chemical materials not normally found in dune sand, also the distribution in the sand of moisture and of oxygen and the varying penetrability of the sand. As the chemical factor seemed

most likely to be the controlling one, some experiments were carried on in the endeavor to clear up this point if possible, and to indicate the nature of the chemicals producing the different results.

Experiments with plants in situ.—The first question to be settled was, can root extension be stimulated by the presentation of nutrient material either to the root tips or along the more mature roots? This was investigated by diffusing Knop's solution from porous cylindrical cups (old atmometer cups in this case) which had been buried in the sand near the roots of growing plants. The cups

Fig. 17.—*P. pumila*, 3 months' seedlings grown in pots: *a*, irrigated with rain water; *b*, with Knop's solution; *c*, grown in rotted barnyard manure and humus.

were located at a distance of 5–10 cm. from large lateral roots near a portion of the root practically without secondary laterals. The cups were filled about 3 times a week with 0.6 Knop's solution and were dug up after 2 months. In every case there was a marked development of laterals in the zone of diffusion of the solution. This was tried with *Prunus pumila* and with *Thuja occidentalis* (figs. 15, 16). The contrast between the old bunched roots and the new slender ones is striking. Controls with distilled water showed no abnormal development.

POT CULTURES WITH NUTRIENT MATERIALS.—Pot cultures with *P. pumila* seedlings were grown in dune sand watered (1) with distilled water, and (2) with Knop's solution, and (3) in a substratum of rotted barnyard manure. The results are shown in fig. 17. While the root systems in (1) and (2) seem rather similar in extension, an examination showed that (2) was more closely branched than (1) and had nearly twice as many absorbing root tips. The striking feature of (3) was the much greater development of the shoot. Attempting to reproduce more closely the natural conditions, a seedling was grown in the laboratory in a pot of sterile dune sand in which a small patch of rotted manure had been included. Here the most marked extension of rootlets appeared under the patch of organic matter in a space stained by leaching from the material above.

POT CULTURES WITH INORGANIC SALTS.—Experiments on the directive influence of inorganic salts have showed so far only indications and suggestions. RUSCHE (32) has already shown differences in extension produced by different salts, and attempts were made to extend his results along the line of directive influence. The technical difficulties of finding suitable seedlings and of presenting the chemicals in solution from one side only, along with the uncertainty as to the direction and extent of diffusion of the solution through the sand, have so far prevented reaching any conclusive results. Indications of a tendency to react in accordance with the sequence of the liatropic series were observed and definite specific differences were also evident.

Discussion and conclusions

The most marked feature of these observations is the varied reactions of different species to apparently identical environmental conditions. These showed sufficient constancy within a species, but with such differences in different species as to suggest that the reactions are specific and that the controlling factor is heredity. Their general character also strongly suggests their relation to a former habitat or condition of growth. It would seem possible that species which had varied in the direction of an extended root system in sandy soil had survived there and had become adjusted to

that environment, and that when germinating in soils richer in nutrients these root systems would be less extended. On the other hand, species which had similarly become adjusted to the richer soils had thereby become dependent on these soils for the development of extended root systems, and were therefore stunted in sandy soils. While a relation to past conditions may not be the true explanation for this specificity, the facts are evident and certainly indicate very definite relations to present conditions. These should be of great value in selecting species for revegetating exposed areas and other localities where the humous content of the soil is slight or unevenly distributed, as well as in the cultural treatment of species which may be regarded as worthy of development for their economic value.

On this basis it would follow that general statements in regard to the root habits of dune plants as a class are dangerous, and that the so-called "dune pioneers" are not all on the same footing; that in fact they should be put into two widely different groups with a series of types occupying intermediate positions. In one group would be the *Prunus pumila* type, which does not have the power of extending root systems widely in pure dune sand, but is stunted and does not reach maturity unless its roots find buried organic matter. In the opposite group would be the *Ammophila* type, which reaches maturity in pure dune sand and whose root system is limited in extension by the presence of decaying plant materials. Similar to *Ammophila* would be *Artemisia*, *Cirsium*, and *Campanula*, with *Calamovilfa* and other grasses, and probably species of *Solidago* and *Aster*. *Lithospermum* is similar ecologically, although with a very different root habit, while *Lathyrus maritima* occupies a peculiar position on account of its relationship to the nodular bacteria. From these observations it would seem that *Ammophila* is the only plant which can reasonably be expected to thrive successfully on the normal dune substratum.

In seeking for the causes of the asymmetric development of root systems observed, it is evident that the only factors to be considered are those working in the soil and exerting an unequal or one-sided influence in the system as a whole. These may be limited to four, namely, moisture, chemicals, oxygen, and density or penetrability

of the soil. In the case of a few plant groups, as the willows, which are recognized as having a hereditary hydrotropic tendency, there will be little question as to the dominant factor, but the evidence in the cases of the species previously described must be considered somewhat carefully.

Taking up first the possibility of moisture as the main factor, it should be noted that below the first few centimeters water is evenly distributed in dune sand, and cannot be regarded as the causal factor in the development of the asymmetric root systems described. It might be assumed that the following of humous layers and dark patches by plant roots should be attributed to the presence of a greater amount of moisture in those patches than in the adjoining sand. Granting the power of humus to absorb greater amounts of water than can the pure sand, it would be difficult to prove that water was held by these patches beyond the quantity required by their increased wilting coefficients. The patches are so small and the medium so unstable that it would be practically impossible to collect from adjoining patches pure samples large enough for accurate determination of their respective moisture contents and wilting coefficients. In the experimental work the adjustment of the water supply was a difficult matter. As a result there were occasional reactions which seemed to point to the influence of moisture, but even in these it could not positively be stated that chemical influence was not the dominant factor with moisture as contributory only.

Approaching the question from the chemical side, we find definite evidence, both from observation and from experimentation, that, with the species considered, variations in chemical solutions produced changes in root development, while variations in water supply produced little or no evidence of such changes in development. Perhaps the most conclusive evidence was found in the case of a patch of humus in a pot culture of *Prunus pumila*. Here the abnormal development of laterals occurred under the patch of humus in a zone stained by leaching of organic matter rather than in the free region on either side, where the moisture content should be the same as in the region under the organic patch.

We find also marked differences in elongation of root systems in the presence of decaying plant parts. Contact with or even coming

into the zone of influence of these plant parts seems to cause elongation of the roots of some species, but inhibition in those of others. The determining factor here might be a direct chemical stimulation, the furnishing of organic material, or in opposite cases the presentation of some injurious or inhibiting chemical product of decay. No deciding evidence has as yet been secured along this line. There were some indications that the roots of seedlings are more sensitive to inhibiting factors than are those of mature plants.

Oxygen.—There was little evidence of unequal distribution of oxygen through the dune substratum. The only exception would be in connection with a high water table, and there the evidence was not conclusive. The slight effects observed were inhibitory in nature, and, in one case at least, referable to destructive action of micro-organisms rather than to a direct reaction of the plant tissue to the absence of oxygen.

Penetrability of the soil.—It is quite possible that the substratum may be more penetrable in some localities, either on account of differing densities of certain layers or of the disintegration of buried plant parts. This would be difficult to prove either way, but there was no evidence of any tendency of the root tips to be turned back by a less penetrable layer when accidentally wandering too near to the borders of a dark layer, as would be the case if the dark layer had been the more penetrable medium. In fact the only observed case of a probable difference in penetrability was that of a soil layer which apparently contained a percentage of wind-blown clay. In this case certain roots were distributed along the upper surface of this layer when normally they might have been expected to pass directly down through it. As already indicated, the difference of penetrability of the moist sand under the soft sand mulch may be the determining factor in the distribution of *Artemisia* laterals. This distribution, however, may also be explained by the difference in moisture content of the two layers of sand.

There is no clear indication as to the method by which the chemical substances act on the root, whether by direct stimulation or by removal of some inhibition; neither are the relative rôles of organic and inorganic substances more than suggested. The cases of marked elongation of roots in one direction would seem to indicate the possibility that roots under certain conditions can make use of

organic matter directly; in other words, that green plants may be somewhat saprophytic in nature. A careful microchemical study of the contents of the root at different points along its length would be necessary in order to get any definite information on this point. It would probably occur only when the root found materials exactly suited to its needs, and would be proportionately greater as the increasing length of the root made transportation to the shoot increasingly difficult.

Under these stimulating influences abnormal lengthening and thickening of roots occur to such an extent as to call in question the value of the common method of estimating root development by measuring the length and weight of roots. In fact, if the explanation of direct local use of organic material of the root be accepted, the abnormally long root may be a detriment instead of a benefit to the plant as a whole. It would seem as though there could be developed some method of evaluating the absorbing power of roots through study of the structure of the different parts of the root system which would give more dependable results than the length and weight method.

The evidence cited emphasizes the unique character of the dune substratum, in that pure dune sand is the only soil in which mineral salts, with the exception of calcium carbonate, are practically absent, and organic matter is so rare and scattered that as a general factor it is practically negligible. This is in strong contrast with many arid deserts in which large quantities of desirable mineral salts are present, needing only the addition of water to make them available for plant use. While irrigation is the main need of many desert stretches, it would not solve the problem of plant culture on a dune area.

Summary

1. The study of the literature shows that the work done on extension and development of root systems has been surprisingly little, in view of the importance of the root in the utilization of moisture and chemicals in the soil. This study also emphasizes the necessity of interpreting the extension of root systems in the light not only of structure and functions, but also of the causes of such extension.

2. The study of the habitat selected shows that dune sand as a substratum for plant growth is almost unique in uniformity of texture and in absence of mineral salts required by growing plants. It is homogeneous chemically, but contains not only old soil layers but minute streaks and patches, apparently of carbonaceous and organic origin, as well as dead plant parts, very unequally distributed.

3. The roots of dune species react differently to the elements of this heterogeneous structure, extension being increased in some species by the buried organic matter, while others seem unaffected or even inhibited by it.

4. These reactions are specific and hereditary, and may reflect the conditions under which the ancestral plants grew. They must be regarded as of great importance in the choosing of species for introduction into conditions where the humous content is uneven.

5. Giving due weight to the possibility of moisture, oxygen content, and penetrability of the sand as influencing factors, the evidence seems to point conclusively to nutrient or at least chemical influence as the cause of variability in symmetry in the extension of roots under dune conditions.

6. Under certain conditions the root apparently utilizes organic matter directly, at the expense of its shoots. Extreme lengthening and thickening of roots occurring under these conditions call into question the value of the common method of estimating plant growth by measuring the length and weight of roots.

NORTHWESTERN UNIVERSITY
EVANSTON, ILL.

LITERATURE CITED

1. ALTEN, H. VON, Wurzelstudien. Bot. Zeit. 67:175–199. 1909.
2. BENECKE, W., Über die Keimung der Brutknospen von *Lunaria cruciata*. Bot. Zeit. 61:19–46. 1903.
3. BENNETT, M. E., Are roots aërotropic? BOT. GAZ. 37:241–259. 1904.
4. BÜSGEN, M., Studien über die Wurzelsysteme einigen dicotyler Holzpflanzen. Flora 95:58–94. 1905.
5. ———, Bau und Leben unserer Waldbaüme. Jena. 1897.
6. CANNON, W. A., Root habits of desert plants. Carnegie Publ. 131. 1911.
7. ———, Treelessness in prairie regions. Carnegie Yearbook 12:71, 72. 1913.

8. CANNON, W. A., Physical relation of roots to soil factors. Carnegie Yearbook 11:61, 62. 1912.
9. ———, Root variation in desert plants. Plant World 16:323–341. 1913.
10. ———, Evaluation of the soil temperature factor in root growth. Plant World 21:64–69. 1918.
11. CANNON, W. A., and FREE, E. E., The ecological significance of soil aëration. Science N.S. 45:178–180. 1917.
12. CRAIG, J., Root killing of forest trees. Iowa Exper. Sta. Bull. no. 44. 1899.
13. DACHNOWSKI, A., Toxic properties of bog water and bog soil. BOT. GAZ. 46:130–143. 1908.
14. ———, Bog toxins. BOT. GAZ. 47:389–405. 1909.
15. ———, Cranberry Island. BOT. GAZ. 52:1–33. 1911.
16. FREIDENFELDT, T., Studien über die Wurzeln krautiger Pflanzen. I. Flora 91:115–208. 1902.
17. ———, Studien über die Wurzeln krautiger Pflanzen. II. Bibl. Bot. 61: 1904.
18. GOFF, E. S., Study of roots of certain perennial plants. Wis. Agric. Exper. Sta. Report no. 14. 286–298. 1897.
19. HELLRIEGEL, H., Beiträge zu den Grundlagen des Ackerbaus. Braunschweig. 1883.
20. HILGARD, E. W., Soils. 1906.
21. HITCHCOCK, A. S., Studies on subterranean organs. I and II. Trans. Acad. Sci. St. Louis 9:1–8, 1899; 10:131–142. 1900.
22. HÖVELER, W., Über die Verwerthung des Humus bei der Ernährung der chlorophyllführenden Pflanzen. Jahrb. Wiss. Bot. 24:283–315. 1892.
23. KING, F. H., Natural distribution of roots in field soils. Wis. Sta. Report 1892, 112; and 1893, 160.
24. KROEMER, K., Wurzelhaut, Hypodermis, und Endodermis der Angiospermenwurzeln. Bibl. Bot. 59:1–151. 1903.
25. NOBBE, F., Über die feinere Verastelung der Pflanzenwurzeln. Landw. Versuchstationen 4:212–224. 1862.
26. NOYES, H. A., TROST, J. F., and YODER, L., Root variations induced by carbon dioxide gas additions to soil. BOT. GAZ. 66:364–373. 1918.
27. PAMMEL, L. H., Weeds of the farm and garden. New York. 1911.
28. PULLING, H. E., Root habit and distribution in the far north. Plant World 21:223–233. 1918.
29. RIGG, G. B., Decay and soil toxins. BOT. GAZ. 61:295–310. 1916.
30. RIMBACH, A., Contractile roots. Abstr. in Bot. Centralbl. 74:209–211. 1898.
31. ROTMISTROV, V., Nature of drought. Relation of root systems to soil and drought. Reviewed in E.S.R. 1914.
32. RUSCHE, E., Beeinflussung der Keimfähigkeit verschiedener Kulturpflanzen durch Salzdüngung. Jour. Landw. 60:303–365. 1912.

33. SCHREINER, O., and REED, H. S., Excretions by roots. Bull. Torr. Bot. Club 34:279–303. 1907.
34. SCHULZE, B., Wurzelatlas. Berlin. 1911.
35. SEELHORST, C. VON, Beobachtungen über die Zahl und den Tiefgang der Wurzeln verschiedener Pflanzen bei verschiedener Düngung des Bodens. Jour. Landw. 50:91–104. 1902.
36. SHEPHERD, J. B., Root systems of field crops. N.D. Station Bull. no. 64. 1905.
37. SHERFF, E. E., Vegetation of Skokie Marsh. Bull. Ill. Sta. Lab. 575–614. 1913.
38. STOHMANN, F., Über einige Bedingungen der Vegetation der Pflanzen. Ann. der Chemie und Pharm. 121:285–338. 1862.
39. TEN EYCK, W. M., The roots of plants. Kansas Exper. Sta. Agric. Coll. Bull. no. 127. 1904.
40. TOLSKY, A., Effect of temperature on the root growth. E.S.R. 14:434.
41. TOTTINGHAM, W. E., Chemical and physiological study of nutrient solutions for plant culture. Physiol. Researches 1:133–245. 1914.
42. TRANSEAU, E. N., Bogs of the Huron Valley. BOT. GAZ. 40:351–418. 1905; 41:17. 1906.
43. TSCHIRCH, A., Über die Heterorhizie bei Dikotylen. Flora 94:69–78. 1905.
44. WATERMAN, W. G., Ecology of Crystal Lake Bar region. Ann. Report, Mich. Acad. Sci. 19:197–208. 1917.
45. WEAVER, J. E., Study of the root systems of prairie plants of southeastern Washington. Plant World 18:227, 273. 1915.
46. ———, Ecology of roots. Carnegie Inst. Publ. (unpublished).
47. WESTGATE, J. M., Reclamation of Cape Cod sand dunes. U.S. Dept. Agric., Bur. Plant Ind. Bull. no. 65. 1904.

SOME PHYLLACHORAS FROM PORTO RICO

F. L. Stevens and Nora Dalby

(WITH PLATES VI–VIII)

The following species were collected by the senior author, and specimens are deposited as is indicated in an article by E. Young.[1] The definition of the genus *Phyllachora* given by Theissen and Sydow[2] is accepted, and only species conforming to their conception of the genus are here listed.

Phyllachora andropogonis K. and H.

On *Imperata contracta:* Maricao, 8875, 8898; Rio Tanamá, 8006; Arecibo-Lares road, 7216.

The spores are much larger than those of *P. graminis*, but agree well with those of *P. andropogonis*. The stromata when young bear many *Septoria*-like conidia.

Phyllachora banisteriae, sp. nov.—Spot none. Stromata numerous, scattered evenly over the leaf, 1–2 mm. in diameter, circular or more often oblong, black, visible from above and below, usually poorly developed in the mesophyll; clypei both above and below, thin, limited to the epidermis. Locules few, large, about 190×270 μ. Asci 8-spored, cylindrical. Spores oblong, hyaline, continuous, 14×35 μ.—Figs. 1 and 2.

On *Banisteria tomentosum:* Vega Baja, 8341.

Conidia, in pycnidia on the stromata, *Septoria*-like. Differs from *P. pestis-nigra* Speg. in many respects.

Phyllachora bourreriae, sp. nov.—Spots none. Stromata circular and black, abundant, scattered irregularly over the leaf, 1–2 mm. in diameter, equally prominent above and below, occupying the mesophyll. Clypeus in epidermis above and below, but slightly exceeding the perithecia. Locules several, globular, about 160 μ in diameter, wall definite. Stroma in the mesophyll loose. Asci cylindrical, 8-spored, 85×9–12 μ. Spores hyaline, 1-celled, 12–16×6–7 μ.—Figs. 3 and 4.

On *Bourreria succulenta:* Vega Alto, 4149 (type); Joyuda, 4770y.

[1] Mycologia 7:143. 1915. [2] Ann. Myc. 13:149. 1915.

Phyllachora canafistulae, sp. nov.—Spots not exceeding the stromata. Stromata mostly large, 2–5 mm., flat, black, visible equally from both sides of leaf, occupying the mesophyll, with many locules. Clypeus more prominent above. Locules globular, 155–170 μ in diameter. Asci cylindrical, 72–99×16–20 μ. Spores oval to ellipsoid, hyaline, continuous, 13–16×6–8 μ. Paraphyses filamentous.—Figs. 5 and 6.

On *Cassia fistula:* Mayaguez, 7022 (type).

Although 2 species of *Phyllachora* are described on *Cassia* (*P. bakeriana* and *P. cassiae* from Brazil), the present form appears entirely distinct from both of them; from the former, which has small stromata and large spores; from the latter, which has small, mostly unilocular, stromata which are limited to the upper part of the mesophyll.

Phyllachora drypeticola, sp. nov.—Spots not exceeding the stromata. Stromata numerous, black, extending through the leaf and visible equally above and below, 1–2 mm. in diameter, in the mesophyll with a thick clypeus on each side. Clypeus not exceeding the stroma. Locules globular or irregular, mostly strictly median, 100–200×125 μ. Asci cylindrical, 8-spored. Spores 17×3.5 μ, continuous, hyaline.—Figs. 7 and 8.

On leaves of *Drypetes* sp.: Rio Tanamá, near Arecibo, 7828 (type). On *Drypetes glauca:* El Gigante, 8558, 8558b; Utuado, 4387; Maracao, 4508, 4472, 1353, 730, 4746, 8558D; Mayaguez, 1834. In certain specimens, as no. 4387, the stromata are less arched of surface and often surrounded by a dead zone of leaf tissue.

This form is especially interesting since the stromata, while bearing perithecia in the mesophyll, often bear conidial layers at the same time, on both its upper and lower surfaces. These beds to the naked eye appear brick-red.

PHYLLACHORA ENGLERI Speg.—Fig. 11.

On *Anthurium scandens:* Las Marias, 430; Monte de Oro, 5712.

Phyllachora gnipae, sp. nov.—Spots numerous, 0.5–1.5 cm. in diameter, surrounding the stromata with an irregularly circular zone, below brown, above brown, or when old blanched. Stromata irregularly circular or angular, 2–5 mm. in diameter, shining black above, dull black below, rough due to the perithecia. Clypei about 30 μ thick in both upper and lower epidermis, extending far

beyond the locules. Locules numerous, variable in size, 190×95 to 380×190 μ, located in the mesophyll. Stroma surrounding the locules poorly developed. Asci cylindrical, 8-spored. Spores oblong, hyaline, continuous, guttulate and granular, obtuse, 18–20×6–7 μ.—Figs. 27 and 28.

On *Gnipa americana:* El. Gigante, 8520.

Differs from *P. laeviuscula* Speg. and *P. guavira* Speg. in character of stroma and size of locules and spores.

PHYLLACHORA GRAMINIS.

On *Eriochloa subglabra:* without number or locality.

Phyllachora heterotrichae, sp. nov.—Spots small, 2–4 mm. in diameter, approximately circular, pale, visible from above and below. Stromata black, irregular in outline, more prominent below, 1–2 mm. in diameter, occupying the whole thickness of the leaf and usually accompanied by increase in leaf thickness, loose in structure. Clypeus epidermal, present above and often extending far beyond the perithecia; usually present also below but less extensive. Locules many, about 110–150 μ in diameter, walls well developed. Asci 8-spored. Spores 13–14×3.5–4 μ, continuous, hyaline.—Figs. 9 and 10.

On *Heterotrichum cymosum:* Villa Alba, 116.

Differs from *P. melastomacearum* Rac. in its much smaller stromata, and from *P. aliena* in its small perithecia.

PHYLLACHORA LATHYRI (Lév.) Theiss. and Syd.—Figs. 12 and 13.

On *Bradburya virginiana:* Tanamá Rio, 7829; Bayamon, 1887; Jayuya, 5991; Manati, 4314; Dos Bocos, 8092; Maricao, 8834. On *Galactia striata:* Jajome Alto, 5644. On undetermined legume: St. Ana, 6651.

Phyllachora mayepeae, sp. nov.—Spots irregularly circular, indefinite, without border, tan or yellow, shading to normal green, 3–15 mm. in diameter, bearing numerous (5–50) circular, black, punctiform stromata which are visible equally above and below, 200–1000 μ in diameter, occupying the mesophyll. Clypei both above and below, but slightly exceeding the stromata. Stromata unilocular. Locules large, globular, with numerous asci. Asci

58–85×18–27 μ. Paraphyses filiform. Spores 9–19×7–12 μ, hyaline, continuous.—Fig. 14.

On *Mayepea domingensis:* Maricao, 785 (type), 775, 731, 765, 4751, 196, 8787, 4720; Mayaguez Mesa, 7471, 7585; Coamo, 148.

The circular tan-colored spots with numerous punctiform stromata are characteristic.

Phyllachora metastelmae, sp. nov.—Stromata shining black, 1–2 mm. wide, 5–15 mm. long, partially encircling the stem, occupying all of the tissues exterior to the wood. Locules about 200 μ in diameter, 120 μ high. Asci 8-spored. Spores cylindrical, hyaline, continuous, 14×5 μ.—Figs. 15 and 16.

On stems of *Metastelma* sp.: El Alto de le Bandera, 8715 (type).

Phyllachora nectandrae, sp. nov.—Spots round or irregular, brown, surrounding the stromata, showing on both sides of leaf. Stromata epiphyllous, black, shining, 1–4 mm. in diameter, slightly raised, scattered or seldom confluent. Locules single or few, nearly globular, located in the mesophyll, 225–500 μ wide, 300 μ deep, side wall thin. Clypeus black, 40–50 μ thick, extending laterally beyond the locules. Paraphyses copious, filiform. Asci cylindrical, 8-spored, 108×10 μ. Spores oblong, 14×5 μ.—Figs. 23 and 24.

On *Nectandra patens:* Maricao, 3608 (type), 8949, 3435, 3730.

Differs from *P. nectandricola* Speg. in having paraphyses and in other details.

Phyllachora ocoteicola, sp. nov.—Spots amphigenous, abundant, angular, 2–4 mm. across; above shining black, below smooth, dull black, with minute hillocks indicating the locules. Clypeus epidermal, apparent both above and below, flat, about 17 μ thick. Stroma of mesophyll consisting merely of scattered mycelial threads. Locules several to the spot, median in the mesophyll, 156–170 μ high, 200–235 μ wide, internal dimensions, wall hyaline, thin. Asci numerous, 8-spored. Spores oblong to cylindrical, 17×54 μ, often somewhat pointed at one end. Paraphyses numerous, filiform, crooked.—Figs. 25 and 26.

On *Ocotea leucoxylon:* Monte Alegrillo, 4768 (type), 4767, 4725; Monte de Oro, 5669; Maricao, 701.

Quite distinct from *P. ocoteae* P. Henn. in shape and character of spot, size of spores, and in other details.

PHYLLACHORA ROUREA Syd., char. emend.—Ascigerous stromata black, numerous, scattered over the leaf, 2–3 mm. in diameter, visible from both sides of the leaf; shining above, dull below, locules 150–240 μ internal diameter, numerous in the stroma. Clypeus above and below extending slightly beyond the locules. Asci numerous, linear, 8-spored, about 100×7–$10\ \mu$, obtuse. Spores uniseriate, hyaline, continuous, $5\times10\ \mu$, oval. Paraphyses scant, filiform.—Figs. 17 and 18.

On *Rourea glabra:* Luquillo, 5447.

The original description by SYDOW was based on Philippine material which contained only conidia. It is very probable that the present specimen is cospecific with that of SYDOW, and the name is therefore retained.

PHYLLACHORA SECURIDACAE P. Henn.—Figs. 19 and 20.

On *Securidaca virgata:* Rosario, 9491; Mayaguez, 7402, 1196, 313; Maricao, 8981.

This species was described from South American material, and our specimens differ as follows from the description: Locules are numerous, not few; the clypeus is distinctly rugose, a striking character that is not mentioned in the description; locules are somewhat larger than as described, and the walls are black, not brown; spores are quite uniformly $10\times5\ \mu$, while the original description gives 15–18×5–$6\ \mu$. Notwithstanding these differences it seems best to report our specimens under this name. Due to an error in host determination 2 of these specimens were reported by GARMAN as *P. perforans* (Mycologia 7:340. 1903).

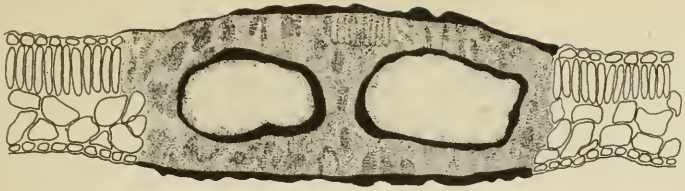
PHYLLACHORA SIMPLEX Starb.—Fig. 21.

On *Coccolobis laurifolia:* Mona Island, 6171, 6433; San Juan, 4060; Bayamon, 394; Monacillo, 9340; Martin Peña, 9311, 9716; San German, 7521, 7519; Tanamá Rio, 7896.

PHYLLACHORA TRAGIAE (B. and C.) Sacc.—Fig. 22.

On *Croton lucida:* Guanica, 356, 6839; Mona Island, 6217, 6211, 6153. On *Croton flavens:* Quebradillas, 9251; Aquadilla, 7253.

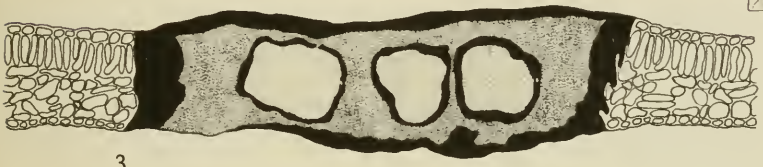
The specimens on the 2 hosts differ slightly, particularly in that the stromata are more frequently unilocular on *C. flavens*, and that on this host the whole structure is more pale, less carbonaceous. Filiform conidia were found associated with the stromata.



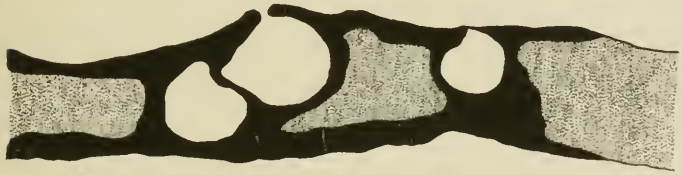
1

2

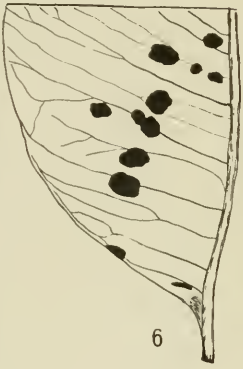
4



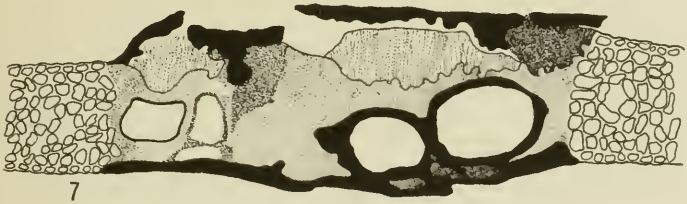
3



5

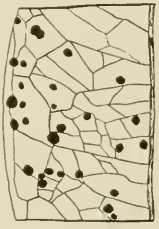


6

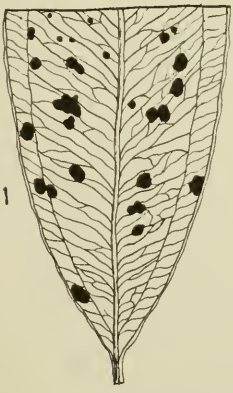


7

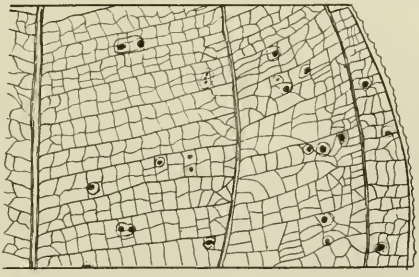
9



8

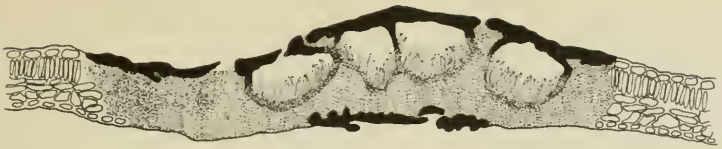


11



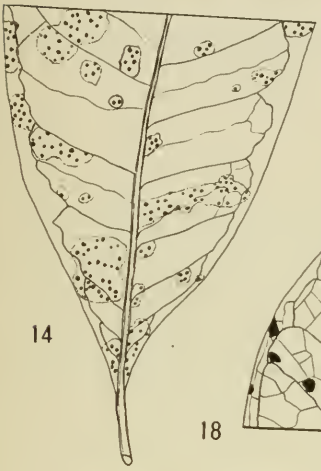
10

STEVENS and DALBY on PHYLLACHORA

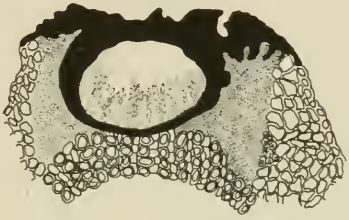


12

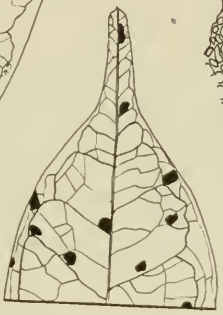
13



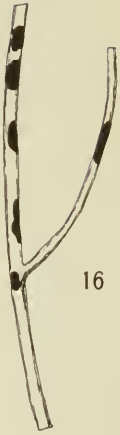
14



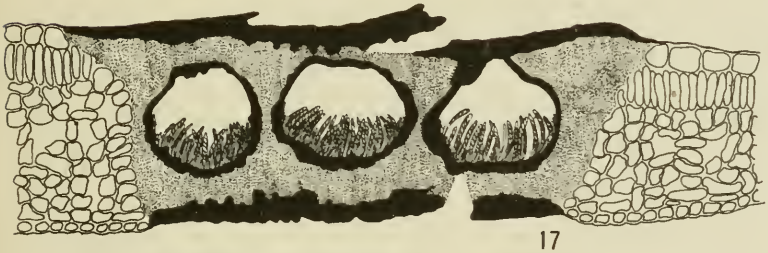
15



18

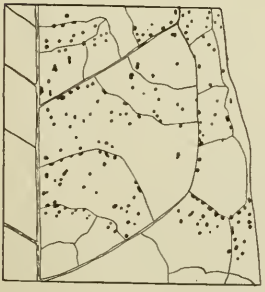


16



17

19

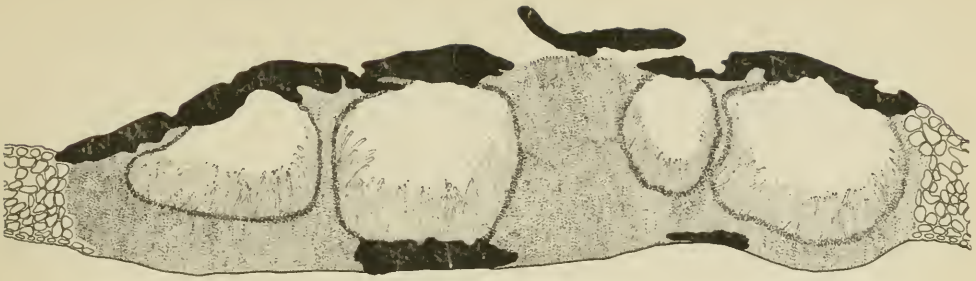


21

22

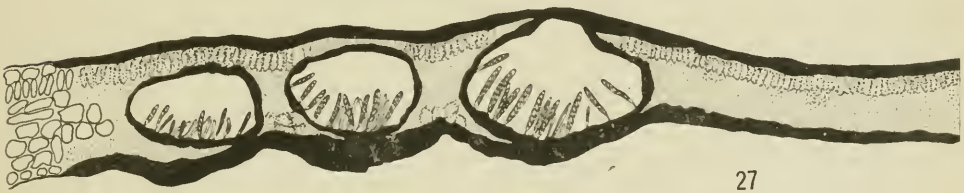
20

STEVENS and DALBY on PHYLLACHORA

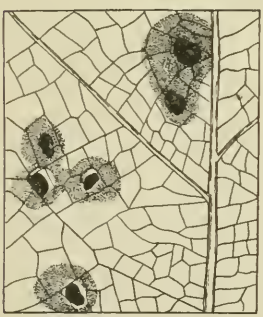


23

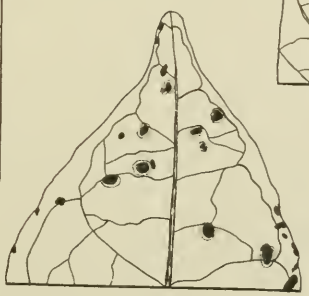
25



27



28



24

26

STEVENS and DALBY on PHYLLACHORA

EXPLANATION OF PLATES VI–VIII

The drawings of the leaf sections were made from permanent slides by the aid of the camera lucida. In general 2 figures are given to each species, a habit drawing of a leaf, or portion of a leaf, and a cross-section of a leaf showing position of locules and clypei in relation to host tissue.

FIG. 1.—*P. banisteriae:* cross-section of leaf, locules median, clypeus poorly developed.

FIG. 2.—*P. banisteriae:* habit sketch.

FIG. 3.—*P. bourreriae:* cross-section of leaf, clypeus well developed; lateral darkening indicates merely a region of denser fungous mycelium, not a true clypeate structure.

FIG. 4.—*P. bourreriae:* habit sketch.

FIG. 5.—*P. canafistulae:* cross-section of leaf, locules not median.

FIG. 6.—*P. canafistulae:* habit sketch.

FIG. 7.—*P. drypeticola:* cross-section of leaf, locules not median; 2 regions of conidiiferous beds shown under upper clypeus.

FIG. 8.—*P. drypeticola:* habit sketch.

FIG. 9.—*P. heterotrichae:* cross-section of leaf; locules numerous, deep, clypei thin.

FIG. 10.—*P. heterotrichae:* habit sketch.

FIG. 11.—*P. engleri:* habit sketch.

FIG. 12.—*P. lathyri:* cross-section of leaf, locules nearer upper surface.

FIG. 13.—*P. lathyri:* habit sketch.

FIG. 14.—*P. mayepeae:* habit sketch.

FIG. 15.—*P. metastelmae:* cross-section of leaf.

FIG. 16.—*P. metastelmae:* habit sketch.

FIG. 17.—*P. rourea:* cross-section of leaf, locules median, clypeus well developed.

FIG. 18.—*P. rourea:* habit sketch.

FIG. 19.—*P. securidacae:* cross-section of leaf, locules large, median, clypei well developed and with rugose surface.

FIG. 20.—*P. securidacae:* habit sketch.

FIG. 21.—*P. simplex:* habit sketch.

FIG. 22.—*P. tragiae:* habit sketch.

FIG. 23.—*P. nectandrae:* cross-section of leaf, clypeus more prominent above.

FIG. 24.—*P. nectandrae:* habit sketch.

FIG. 25.—*P. ocoteicola:* cross-section of leaf, locules median, clypeus above and below.

FIG. 26.—*P. ocoteicola:* habit sketch.

FIG. 27.—*P. gnipae:* cross-section of leaf, clypei well developed above and below.

FIG. 28.—*P. gnipae:* habit sketch.

APPARATUS FOR THE STUDY OF PHOTOSYNTHESIS AND RESPIRATION

W. J. V. Osterhout

(with one figure)

Two recent papers have dealt with methods of studying photosynthesis. The first of these[1] discussed photosynthesis in land plants, while the second[2] dealt with aquatics. It seemed to the writer that these methods might be combined, and some experiments were made for this purpose. The outcome was a simple method for the study of photosynthesis in land plants.

The apparatus consists of a large tube (a lamp chimney will serve) closed at the bottom by a stopper through which passes a tube (*A*, fig. 1) of Pyrex glass. Through the stopper at the upper end passes the neck of an atomizer bulb (*B*) with an opening at *C* for the intake of air.[3] To the neck of the bulb is attached a tube of Pyrex glass which extends to within an inch of the bottom of the tube *A*.

Plants[4] are placed in the chamber with their stems dipping in water contained in a small beaker. By means of the tubes *D* and *E* any desired amount of CO_2 may be run into the chamber. When this is finished the bulb is repeatedly squeezed so as to force the gases in the chamber to bubble through the liquid contained in the tube *A*. This liquid consists of distilled water, to which has been added[5] an indicator which is sensitive to CO_2. As the gas bubbles through the liquid[6] the color of the indicator changes.

[1] Osterhout, W. J. V., Amer. Jour. Bot. 5:105. 1918.

[2] Osterhout, W. J. V., and Haas, A. R. C., Science N.S. 47:420. 1918.

[3] This form of bulb can easily be obtained in the drug trade.

[4] *Tradescantia* may be recommended for this purpose, especially kinds without stripes on the leaves.

[5] The choice of indicator depends on the amount of CO_2 introduced into the chamber; for most purposes phenolsulphonephthalein will prove useful. At the start of the experiment the PH value should be such that a slight change in CO_2 will alter the color of the indicator.

[6] The bulb does not permit the entrance of air from the outside; it merely causes a circulation of the gas within the chamber.

The bubbling must be continued until the color becomes constant. When this is achieved we know that equilibrium has been established between the CO_2 in the liquid and that in the chamber.

In order to determine when the color of the indicator has become constant it is compared with a series of buffer solutions having the same concentration of indicator and contained in Pyrex tubes of the same size, as described in a previous article.

The plant is now exposed to sunlight.[7] After exposure the gas is again bubbled through the liquid. If the plant has taken CO_2 from the air it will be evidenced by the change in the color of the indicator, which will show a greater degree of alkalinity than before. From the amount of change in alkalinity the change in tension of CO_2 can be calculated; or the indicator may be calibrated.[8]

For qualitative results the calculation or calibration is not necessary. In the opinion of the writer, leaves of land plants are not suited to quantitative investigations on photosynthesis, since on exposure to sunlight their temperature (and consequently the rate of photosynthesis) fluctuates greatly (as much as 10° C. in half an hour). This difficulty may be obviated by using suitable aquatics.

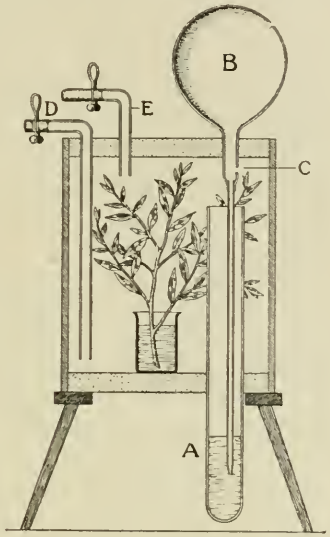


FIG. 1.—Apparatus for measurement of photosynthesis and respiration: plants are placed in the chamber; by means of bulb *B* gas in chamber is bubbled through indicator contained in tube *A;* intake of bulb is at *C* so that no air enters from outside; valve (not shown in figure) prevents air from passing out through *C;* changes in color of indicator show changes in tension of CO_2.

For class demonstration it is not necessary to have a tube projecting through the lower stopper. The apparatus may be

[7] The rise of temperature which occurs in sunlight tends to force gas out through the joints, which should therefore be made tight or sealed with water.

[8] For methods see HENDERSON, L. J., and COHN, E. J., Proc. Nat. Acad. Sci. 2:618. 1916; MCCLENDON, J. F., GAULT, C. C., and MULHOLLAND, S., Publ. no. 251, Carnegie Inst. 1917. p. 21.

simplified by using an ordinary bottle and placing in it a small vial containing indicator, into which dips the tube which is attached to the bulb. It is always advisable to have a control in the light without a plant and a control in the dark containing a plant similar to the one used in the experiment. For the measurement of respiration the procedure is the same, except that no CO_2 is introduced at the start and that phenolsulphonephthalein or a similar indicator is employed. For quantitative work on respiration it may be desirable to have the bubbling go on without interruption. This may be accomplished by an excentric wheel driven by a small motor, and so arranged that at every revolution it compresses the bulb sufficiently to send a few bubbles through the liquid.[9]

Whenever it is desirable to remove the accumulated CO_2 the tubes *D* and *E* are opened and a current of air is run through the chamber (by means of an attached aspirator or syringe). The tubes *D* and *E* are then closed and the gas is bubbled through the indicator until equilibrium is established. The apparatus is then ready for starting a new experiment.

In the same manner air charged with volatile substances (ether, chloroform, etc.) may be introduced in order to study their effect upon photosynthesis and respiration (substances having a pronounced acid or alkaline reaction or a strong buffer effect are unsuitable for this purpose).

Summary

The photosynthesis and respiration of land plants may be studied by placing them in a chamber in which the gas can be made to bubble through an indicator. The changes in the color of the indicator indicate the changes in the tension of CO_2.

The method is so simple and convenient that it is adapted to classroom demonstration as well as to investigation.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

[9] See OSTERHOUT, W. J. V., Jour. Gen. Physiol. 1: 17. 1918.

BRIEFER ARTICLES

A CORN-POLLINATOR

(WITH ONE FIGURE)

In some corn-breeding experiments conducted during the summer of 1918 at Col. GEORGE FABYAN'S Riverbank Research Laboratories, Geneva, Illinois, the writer employed a simple pollination apparatus which has proved very satisfactory. An ordinary thistle tube is filed off 3–4 inches from the bulb and given a bend of 90° (fig. 1, *B*). The

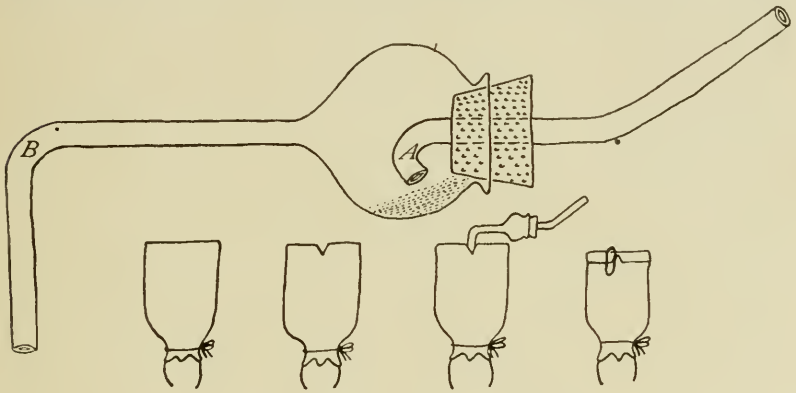


FIG. 1.—Corn-pollinator: description in text

bulb is fitted with a one-hole rubber stopper, through which is passed a glass tube with two bends (fig. 1, *A*). Silks and tassels are covered, as is customary, with manila bags of the ordinary type (single acute angle at closed end). The stopper is removed from the pollinator and into the bulb is shaken an appropriate amount of pollen from the tassel-bag. A small notch is then cut in the closed end of the silks-bag. Through this aperture the thistle-tube end of the pollinator is immediately inserted; the operator blows at the other end of the apparatus; and a dense cloud of pollen is discharged over the silks. The end of the silks-bag is then folded over and held secure with a wire clip (see the series in fig. 1). Thus pollination can readily be repeated, although this is quite unnecessary if the original pollination was made at the time of good pollen and receptive silks. Repetition may be desired, however,

in experiments on the condition of pollen grains themselves or the effect of diverse pollination.

Such an apparatus has three fundamental advantages over any other method that the writer has seen described: (1) foreign pollen is absolutely eliminated, so far as the writer's experience goes; (2) the cloud of pollen is in violent circulation when it strikes the silks, insuring rather complete pollination, always provided that the pollen and silks are both in good condition; (3) it is a relatively "fool-proof" method, so that pollination can be allotted among unskilled assistants.

Certain disadvantages may be considered. (1) *Expense.*—The apparatus can be made up for about 15 cents, and the number of pollinators need not be great. With a given "load" of pollen 4 or more ears may be pollinated. Repeated loadings may be made as long as pollen from a given tassel is desired. The pollinator is then opened, easily washed in 95 per cent alcohol, wiped out with cotton, and dried within 20 minutes, provided there is standing alcohol in none of the parts. The writer usually used about 20 pollinators and then washed them all. (2) *Breakage.*—This is not a problem provided the bent end at *A* (fig. 1) is not long enough to strike the side of the bulb when the stopper is forced in. (3) *Clogging.*—If the bend at *B* (fig. 1) is a clean one, even fragments of tassels contained in the pollen load will pass through readily. This also will depend on the bore of the thistle tube, but the writer encountered practically no difficulties of this sort. (4) *Loading.*—This is the only aggravating feature of the method. It is not a problem provided the operator can afford to sacrifice the tassel, as the writer usually did. The severed tassel is then shaken within the bag, from which pollen is readily poured into the pollinator. Frequently, however, it is desirable to save the tassel. In that case the operator may pull the bag off the tassel and replace after loading. This obviously endangers a wholesale loss of pollen and an exposure of the tassel for a moment. A much better method is merely to loosen the string around the tassel-bag and load the pollinator from one corner of the bag's mouth. This method was employed with considerable success, but it is rather awkward at best and impossible for 12-foot corn. A much more clean-cut method would be to save pollen (rather than tassels) in dry phials, but this multiplies apparatus. With all the drawbacks of loading, however, even an unskilled operator finds little difficulty in using this corn-pollinator.—MERLE C. COULTER, *University of Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Life and letters of Hooker

No botanist needs an introduction to Sir JOSEPH HOOKER, so far as his botanical activities are concerned, but to come into personal touch through his letters to his intimate friends is an unusual opportunity. LEONARD HUXLEY,[1] the son of HOOKER'S close friend, has provided this opportunity by editing materials collected and arranged by Lady HOOKER. HOOKER'S long life, of nearly a century, spanned the revolution in biology, and his intimate relation with DARWIN and his work makes many of his letters a description of the theory of natural selection in the making.

Especially interesting to American botanists are his letters in reference to his visit to the United States in 1877, during which his long-time friend ASA GRAY conducted him across the continent. HOOKER'S impressions of the United States and of its flora make most interesting reading.

No brief review can do justice to the wealth of material in such a collection of letters. HOOKER was fond of writing letters to his intimates, and as a result these volumes seem to make one acquainted with the man. When to the high qualities of the man there is added the importance of the times in which he lived and worked the interest is multiplied.—J. M. C.

MINOR NOTICES

North American flora.—The first part of volume 24 includes most of the genera of the tribe Psoraleae (Fabaceae) by RYDBERG, the genus *Eysenhardtia* being monographed by PENNELL. The tribe, as presented, includes 19 genera, 7 of which are described as new. *Hoita* (11 spp., 4 new), *Rhytidomene* (1 sp.), *Psoralidium* (14 spp., 1 new), and *Pediomelum* (22 spp., 7 new) are segregated from *Psoralea*, which is left with a single species (*P. fruticans*). The revival of *Orbexilum* Raf. also accounts for 8 other species of *Psoralea*. *Psorobatus* (2 spp.), *Psorodendron* (12 spp., 2 new), and *Psorothamnus* (8 spp., 4 new) are segregates of *Dalea*. *Amorpha* seems to retain its identity, with 23 spp., 5 of which are new. *Eysenhardtia* is doubled in representation, 7 of the 14 species being new. The contribution is a complete upsetting of the former presentations of Psoraleae.—J. M. C.

[1] HUXLEY, LEONARD, Life and letters of Sir JOSEPH DALTON HOOKER. 2 vols. 8vo. pp. 546 and 569. New York: D. Appleton & Co. 1918.

NOTES FOR STUDENTS

Infection as related to humidity and temperature.—The importance of humidity and temperature in relation to infection has long been known to plant pathologists. The problem, however, has been taken up once more by LAURITZEN[2] in a study of diseases caused by *Puccinia graminis* on wheat, *Ascochyta fagopyrum* on buckwheat, and *Colletotrichum lindemuthianum* on red kidney bean.

The apparatus used consisted of two double-walled chambers, each provided with heating coils, thermostats, and thermoelectric connections to furnish heat and power to run the fans. The outer wall of each chamber was made of glass, the inner of wood except the lids (which were of glass), the wood portions being coated with paraffin of a high melting point to prevent absorption of water. Evaporation surface for the control of humidity was furnished by pans containing either water or saturated salt solutions. No attempt was made to control light, after experiments designed to test its relation to infection had given negative results. Carbon dioxide is thought to have been of little or no importance in the experiments, since the experimental chambers were not so tight but that gaseous exchange was possible between inside and outside whenever temperature variations occurred. Pure line strains were used in the case of all 3 of the fungi, but only of wheat in the case of the hosts. Plants of the same age and spores of the same age were used as far as possible, although there was some unavoidable variation in both cases.

The author uses as a criterion by which to judge the effect of temperature and humidity the percentage of plants infected out of the total number exposed to infection. He thus avoids most of the objections which have been made to the criteria used by other workers. Where they have measured the effect of temperature and humidity (on the fungus alone) by the rate of extension of the germ tube and development of the mycelium, by the time required for germination of spores, or by the percentage of germination, he measures it by a criterion which takes account of fungus and host as well.

The lower temperature limit for infection of wheat by *Puccinia* was found to be about 42° F.; of buckwheat by *Ascochyta* about 45° F.; and of bean by *Colletotrichum* about 57° F. The upper temperature limits in the 3 cases were respectively 80, 100, and 80° F. The range of humidity (relative) for infection of wheat varied between 92 and 100 per cent; of bean, between 92 and 100 per cent; of buckwheat, between 90 and 100 per cent. In no case did he find the temperature ranges for host and parasite identical. The temperature range for growth of bean and wheat is wider than that for the germination of spores of an infection by *C. lindemuthianum* and *P. graminis*. The upper temperature limit for the growth of bean is higher than that for germination of spores of and infection by *C. lindemuthianum*, a fact which has made possible

[2] LAURITZEN, J. T., The relation of temperature and humidity to infection by certain fungi. Phytopathology **9**: 1–35. 1919.

the control of anthracnose in the southern states. The exact temperature range for *Ascochyta* and buckwheat was not determined.

Results obtained by other workers have shown that there is no definite optimum for infection, but that there is a wide range of temperature in which, providing some other factor does not interfere, the number of infections taking place does not vary much if the plant is exposed to infection for a sufficient length of time at the lower temperatures. These earlier results are borne out by the work of LAURITZEN. He concludes, therefore, that in considering control measures we must note not only the temperature which seems favorable to infection, but also the length of time for which the plant has been exposed.

The results he obtains from a study of the effect of humidity on infection lead him to point out that "the absence of certain diseases in semi-arid and arid regions where agriculture has been practised for long periods of time may be due in part to the small moisture content of the air," and that "seasonal variation in the moisture content of the air plays an important part in determining the amount of disease that develops." Because of the rather general belief that a film of moisture on the leaf surface favors infection, it is interesting to note the conclusion reached by the author that such a film is not essential. He considers it proved that, within certain limits of humidity, the spore is able to absorb sufficient water for germination, at first by imbibition and later by osmosis. He suggests also that in depressions of the leaf surface, especially above the stomata, the humidity may be high enough to make germination possible.—D. H. ROSE.

Life history and sexuality of Basidiomycetes.—Miss BENSAUDE[3] has investigated *Coprinus fimentarius*, *Armillaria mucida*, and *Tricholoma nudum*. The work includes two phases: (1) the morphology and cytology of the mycelia, and (2) the results obtained from the study of the single spore cultures of *C. fimentarius*.

The mycelia of the 3 species were obtained from germinating spores as well as from material collected in the field. The author accepts FALCK's classification of the mycelia into primary, secondary, and tertiary forms. The claim is made that the first few days after the germination of the spores the resulting mycelia belong to the primary class, in which the hyphae are partitioned off into cells which contain from one to many nuclei. These uninucleate cells may give rise to varying numbers of uninucleate oidia. Disarticulated hyphal cells, which she calls "pseudoidia," are also formed which, like true oidia, may germinate. The nuclei in the germ tubes divide amitotically. Cross-walls with clamp connections never appear in the hyphae of the primary mycelia. Miss BENSAUDE grew single spores of *C. fimetarius* in pure cultures, and succeeded in isolating 10 single spores. Of these, 4 germinated, and in 2

[3] BENSAUDE, MATHILDE, Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. Dissertation. pp. 156. *pls. 13. figs. 30.* Nemours. 1918.

cultures primary mycelia were obtained which did not produce carpophores. When parts of each mycelium were mixed in a culture, a secondary mycelium appeared and fruit bodies were produced. The chief method of bringing about the plasmogamy is through the union of a hyphal cell of one thallus with an oidium from another thallus. Miss BENSAUDE concludes that the "dicaryon" in *C. fimetarius* is formed following plasmogamy between cells coming from 2 different thalli.

The transformation of a primary mycelium into a secondary mycelium is very difficult to observe. This is brought about by the anastomosis of 2 hyphal cells of different thalli in *C. fimetarius*. The fusion of 2 such cells (plasmogamy or pseudogamy) introduces the cytoplasm and nucleus or nuclei of one cell into the other, which results in the establishment of a binucleate cell. If 2 cells unite which have more than 2 nuclei in common, all disintegrate but 2. The uninucleate oidium may fuse with a hyphal cell, and this is a very common means of bringing about the initial binucleate condition of the cell.

Each cell in these secondary hyphae is binucleate, constituting a "dicaryon." Conjugate nuclear division occurs in these hyphae as a rule in the apical cell, although intercalary cells divide occasionally. At the time of division the 2 nuclei move to the middle of the cell, and the actual process of cell division is preceded by the formation of a protuberance which is to form a clamp. One of the nuclei, which Miss BENSAUDE calls (+), on the basis of her results with single spore cultures, enters this very short branch, and the (−) nucleus remains at about the same level in the mother cell. Spindles are formed and conjugate nuclear division takes place. One of the (+) daughter nuclei goes back into the mother cell, and the other goes to the apex of the young clamp. A cross-wall cuts off the beak cell from the mother cell. Of the 2 (−) daughter nuclei, one goes to the apical part of the mother cell and the other to the basal part, and a cross-wall is formed at the level of the young clamp, dividing the cell into an apical portion with (+) and (−) daughter nuclei and a basal cell with only the (−) daughter nucleus. The little beak now fuses with the basal cell, and its nucleus passes into this cell, so that it also becomes binucleate. Very often the apex of the beak fuses with the mother cell before nuclear division takes place.

Reversion of secondary to primary mycelium occurs, in which case a uninucleate cell appears among binucleate cells. No clamps are found on the cross-walls of this cell, and these uninucleate cells may bear oidia.—MICHAEL LEVINE.

A new conception of sex.—JONES[4] presents a conception of sex which is quite unorthodox, but at least it furnishes considerable food for thought. This author sees in fertilization an "attack" of a "parasitic" male gamete

[4] JONES, W. N., On the nature of fertilization and sex. New Phytol. 17:167–188. 1918.

upon a female gamete well stocked with food reserves, the stimulus of fertilization being similar to that induced in gall formation. The first sperm "vaccinates" the egg and renders it immune to other sperms. Many further analogies are drawn between sex and parasitism or symbiosis. More interesting is his distinction in higher forms between "sex" and "gender." Sex is purely sporophytic, determined in Mendelian manner by chromosome equipment; "male" signifies "microspore-producing," "female" signifies "megaspore-producing." "Gender" is gametophytic and is lodged in the cytoplasm; the nature of the cytoplasm may show gradations between the two extremes of "androplasmic" (or sperm-producing) and "gynoplasmic" (or egg-producing). Thus in a homosporous pteridophyte the spore is still diploid with reference to gender, which is differentiated later. Gradually the androplasm begins to dominate in some cells, gynoplasm in others, until at last the cells are sufficiently unlike to fuse again. Which kind dominates in a particular region may be tied up with nutrition. In heterosporous forms "the archesporial tissue of the anthers is predestined normally (chromosomes) to develop into microspores, an environment which favors the dominance of androplasmic protoplasm." To explain hermaphroditic spermatophytes the author states that "the production of anthers or ovaries is a sex or somatic (Mendelian) characteristic, which may show somatic segregation like other somatic characters." Carrying these ideas over to man, an effeminate man would be produced from an x zygote in which gynoplasm dominated, a masculine woman from a $2x$ zygote in which androplasm dominated. The author believes that many of the characteristics popularly associated with one sex only are in reality the common property of both sexes, although in one they may perhaps be limited in their expression.—Merle C. Coulter.

Mineral absorption in spinach.—In attempting to demonstrate a causal relation between spinach blight and universal malnutrition, True and his colleagues[5] subjected spinach to very heavy applications of various fertilizers, both singly and in mixtures. As high as 1500 lbs. per acre of NaCl, $NaNO_3$, and Na_2SO_4, 6 tons of $CaCO_3$, 2 tons of $MgCO_3$, 2000 lbs. of acid phosphate, 4000 lbs. of complete fertilizer, and 40 tons of manure were used. Although failing to throw any light on the origin of the blight, the results contribute to our knowledge of mineral absorption by plants. The total ash, and each of its constituents with the exception of manganous oxide, was always greater in amount in the leaves than in the tops. The ash elements fall naturally into two groups: (1) those that are present in quantities that show relatively little variation whatever be the chemicals added to the soil (CaO, MgO, P_2O_5, SO_3, MnO, Al_2O_3, and Fe_2O_3); and (2) those which show great fluctuations in the quantity present (SiO_2, K_2O, and Na_2O). The elements of the first group

[5] True, R. H., Black, O. F., and Kelly, J. W., Ash absorption by spinach from concentrated soil solutions. Jour. Agric. Res. 16:15–25. 1919.

were evidently absorbed in the required quantities irrespective of what was offered in excess. Those of the second group varied widely, sometimes with an increase of the ion offered in excess, as in the case of Na_2O, and sometimes with an increase of some other element, as in the case of SiO_2 in the plots receiving $CaCO_3$ and acid phosphate. The soda-potash ratio was subject to extreme variation, but was always greater than 1 in both tops and roots. There were indications that sodium may partly replace potassium in function in spinach, since the percentages of the two usually varied in the reverse order. The writers suggest that NaCl as a fertilizer for other crops might serve as a potash sparer. There was always more magnesia than lime present, except in the plots receiving a heavy treatment of $CaCO_3$, which suggests the possible practical value of magnesium salts as fertilizers for spinach.—J. J. WILLAMAN.

Permeability.—A new working hypothesis as to the nature of permeability and changes in permeability of protoplasm, which seems to the reviewer less objectionable than any yet proposed, is offered by FREE.[6] Protoplasm is considered as a colloidal system of at least two phases, differing from one another mainly in the proportion of water each contains, and arranged as colloidal globules in a colloidal medium. These phases are supposed to exhibit interchange of water, so that globules may decrease in size by giving up water to the medium, which gains in size; or, vice versa, globules may increase in size by receiving water from the medium, which thus becomes thinner and thinner as the globules enlarge.

The medium is considered the important phase from the standpoint of permeability changes, as it is continuous. Anything that can dissolve in the medium should be able to penetrate. Water undoubtedly penetrates both phases. Anything which tends to increase the size of the globules at the expense of the medium is conceived to decrease the permeability of the protoplasm; conversely, things tending to decrease the globules are conceived to increase its permeability. Semipermeability is related to a very thin medium between the globules. Any reagent increasing thickness of the medium at the expense of the globules should decrease semipermeability if this conception is correct. Antagonism would be explained by the effect of the antagonistic element or ion on the globules, enlarging them so that the medium is too thin to permit entry of the toxic element. As a working hypothesis it has some advantages over any other hypothesis which has been proposed. It should stimulate research designed to test its merits, for definite testing seems quite possible.—C. A. SHULL.

Self-sterility.—EAST and PARK have already demonstrated[7] that self-sterility in tobacco is heritable, and that cross-sterility depends upon likeness

[6] FREE, E. E., A colloidal hypothesis of protoplasmic permeability. Plant World 21:141–150. 1918.

[7] BOT. GAZ. 66:461–462. 1918.

in certain significant germinal factors. Thus if *A* is sterile with *B* and *C*, *B* will be sterile with *C*. Shifting now[8] to the physiological technique, they discover that sterility is tied up with the rate of pollen-tube growth. In cases of selfing or cross-sterile pollinations, the pollen not only germinates successfully but develops a normal pollen tube. This tube grows through the style at a uniform rate, but fails to reach its goal before the flower decays. In cases of cross-fertility the pollen tube grows at a progressively increasing rate. The logical conclusion is that self-sterility is not due to the presence of inhibiting substances, but rather to the absence of accelerating substances (catalyzers produced by the pollen-tube nucleus in compatible crosses, and only local in their effect). At the wane of the flowering period self-sterility and cross-sterility may be replaced by "pseudo-fertility." This is explained by the breaking down of the stylar tissue, so that own pollen tubes grow at a uniformly greater (not accelerated) rate.—MERLE C. COULTER.

Photometry.—The probability that a solution of uranium acetate and oxalic acid may be used successfully as a chemical photometer in physiological researches involving the measurement and comparison of light intensities is indicated by some experiments by RIDGWAY.[9] The solution used consisted of 1 per cent uranium acetate and 5 per cent oxalic acid in aqueous solution mixed in the proportion of 1:4. In various tests designed to compare the oxalic-acid–uranium-salt photometer with the Callendar pyrheliometer, the chemical photometer gave results in general agreement with the pyrheliometer, even though the 2 instruments involve different portions of the solar spectrum. If the instrument and methods of using the solution can be reliably standardized, the inexpensiveness of the materials, ease of taking readings, accuracy of determinations, and its automatic integration for variable conditions of light during exposure will make it an excellent instrument for extending our knowledge of the influence of light as related to life processes in plants and animals.—C. A. SHULL.

Nitrogen fixation.—Another contribution from the Missouri Botanical Gardens on the subject of nitrogen fixation by lower organisms deals with the growth of *Azotobacter* in synthetic media. ALLEN[10] believes that most of the discrepancies in the results of previous investigators can be explained on the basis of the phosphorus requirements of the organism and the reaction of the medium. He proves this in a fairly satisfactory way by means of the following factors: (1) when $CaCo_3$ is used to maintain a proper P_H in the media, the

[8] EAST, E. M., and PARK, J. B., Studies on self-sterile plants. II. Pollen-tube growth. Genetics 3:353–366. *figs.* 3. 1918.

[9] RIDGWAY, CHARLES S., A promising chemical photometer for plant physiological research. Plant World 21:234–240. 1918.

[10] ALLEN, E. R., Some conditions affecting the growth and activities of *Azotobacter chroococcum*. Ann. Mo. Bot. Gard. 6:1–44. 1919.

phosphates are precipitated out quantitatively, and scant growth occurs; (2) when glycerol phosphate is used, the phosphorus stays in solution and better growth results; (3) the use of protective colloids (agar and potassium silicate) to prevent precipitation is accompanied by beneficial results; (4) mechanical agitation of the cultures greatly improves the growth by hastening the solution of $CaCO_3$, and thus maintaining the proper reaction. In the course of the work an all-glass apparatus for the determination of nitrogen was devised.[11]—J. J. WILLAMAN.

Distribution of dissolved oxalates in phanerogams.—MOLISCH[12] finds dissolved oxalates appearing rather generally distributed in phanerogams. All investigated species of the following families bear much dissolved oxalate: Polygonaceae, Chenopodiaceae, Amarantaceae, Aizoaceae, Begoniaceae, Melastomaceae, Oxalidaceae, Cannaceae, and Marantaceae. While in most cases this chemical character, like many other chemical characters, runs by families, this is not always the case. In certain families some genera are very rich in dissolved oxalates, while other genera contain little or none; this is true of Commelinaceae and Cactaceae.—WM. CROCKER.

Water movements in plants.—RENNER[13] answers NORDHAUSEN'S criticism (Ber. 1916) of his earlier work (Flora, 1911) on water movement in plants, and gives a number of experiments to confirm, in the main, his earlier generalizations. He also gives a brief statement on the "saturation deficit" and the "energetics of water movement" in plants.—WM. CROCKER.

Turgor and osmotic pressure.—THODAY[14] gives a simple elementary analysis of turgor, osmotic pressure, and saturation deficit relations of plant cells and the conditions that lead to the movement of water from cell to cell in the plant. The article ought to do much to clear up the confusion in reference to this field.—WM. CROCKER.

Hydnaceae of North Carolina.—COKER[15] has published a monograph of the Hydnaceae of North Carolina, illustrated by numerous excellent photographic plates. Six genera are presented, represented by 29 species, 2 new species being described in *Hydnellum* and 1 in *Phellodon*.—J. M. C.

---

[11] ALLEN, E. R., and DAVISSON, B. S. An all-glass nitrogen apparatus. Ann. Mo. Bot. Gard. 6:45–48. 1919.

[12] MOLISCH, HANS, Über den Microchemischen Nachweis und die Verbreitung gelörter Oxalate im Pflanzenreiche. Festschrift zum ERNST STAHL. pp. 60–70. Jena. 1918.

[13] RENNER, O., Versuche zur Mechanik der Wasserversorgung. Ber. Deutsch. Bot. Gesells. 36:172–179. 1918.

[14] THODAY, D., On turgescence and the absorption of water by the cells of plants. New Phytol. 17:108–113. 1918.

[15] COKER, W. C., The Hydnums of North Carolina. Jour. Elisha Mitchell Sci. Soc. 34:163–197. *pls. 29*. 1919.

The University of Chicago Science Series

EDITORIAL COMMITTEE

ELIAKIM HASTINGS MOORE, *Chairman*

JOHN MERLE COULTER ROBERT ANDREWS MILLIKAN

The Evolution of Sex in Plants. By JOHN MERLE COULTER. x+140 pages, 12mo, cloth; $1.00, postpaid $1.10.

Individuality in Organisms. By CHARLES MANNING CHILD. x+212 pages, small 12mo, cloth; $1.25, postpaid $1.40.

The Origin of the Earth. By THOMAS C. CHAMBERLIN. xii+272 pages, small 12mo, cloth; $1.50, postpaid $1.65.

Finite Collineation Groups. By HANS F. BLICHFELDT. xii+194 pages, small 12mo, cloth; $1.50, postpaid $1.60.

A Chemical Sign of Life. By SHIRO TASHIRO. x+142 pages, small 12mo, cloth; $1.00, postpaid $1.10.

The Biology of Twins. By HORATIO HACKETT NEWMAN. x+186 pages, small 12mo, cloth; $1.25, postpaid $1.35.

Food Poisoning. By EDWIN OAKES JORDAN. viii+116 pages, small 12mo, cloth; $1.00, postpaid $1.10.

The Electron: Its Isolation and Measurement, and the Determination of Some of Its Properties. By ROBERT ANDREWS MILLIKAN. xii+268 pages, small 12mo, cloth; $1.50, postpaid $1.65.

The Living Cycads. By CHARLES JOSEPH CHAMBERLAIN. xiv+172 pages, small 12mo, cloth; $1.50, postpaid $1.60.

Problems of Fertilization. By FRANK R. LILLIE. xii+278 pages, small 12mo, cloth; $1.75, postpaid $1.90.

VOLUMES PLANNED OR IN PREPARATION

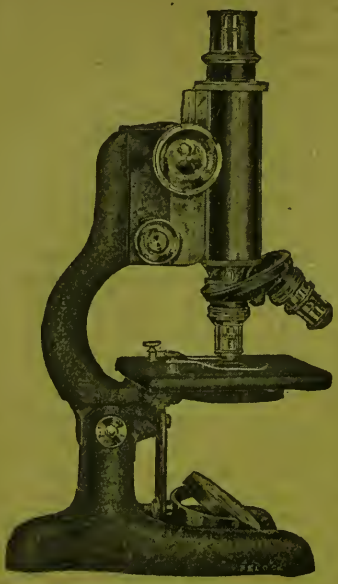
Black Body Radiation. By CHARLES E. MENDENHALL.

Mechanics of Delayed Germination in Seeds. By W. CROCKER.

The Rigidity of the Earth and of Materials. By A. A. MICHELSON.

Linear Integral Equations in General Analysis. By ELIAKIM HASTINGS MOORE.

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - ILLINOIS



FS 2 Microscope—Price $40.75

Bausch and Lomb Microscopes

are again in the market—backed by the prestige which attaches to successful war service in any significant capacity.

Our release from this service is of course gradual. As the demands made upon us by the government are lessened from month to month, however, we are enabled to increase deliveries to our other patrons.

Write for complete catalog of our revised microscope line and inform us regarding your requirements.

Bausch & Lomb Optical Co.
412 ST. PAUL STREET ROCHESTER, N.Y.
New York Washington Chicago San Francisco

Leading American Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

FINE INKS AND ADHESIVES
For those who KNOW

Higgins'

- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

At Dealers Generally

CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street Brooklyn, N.Y.

It Gives the Typist More Time to Type

These three things—*inserting the paper, typing, line spacing,* are all the typist has to do when she operates the

SELF-STARTING REMINGTON

Hand settings of the carriage, which are necessary on all other typewriters, are eliminated absolutely—and that means a time saving of 15 to 25 per cent in transcribing business letters. The Remington Self-Starter, or automatic indenter, gives the typist more time to type. That means a bigger day's work with no more effort; bigger typewriter value at no greater price.

REMINGTON TYPEWRITER COMPANY
(Incorporated)
374 Broadway New York

Volume LXVIII Number 2

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

AUGUST 1919

A Sporangiophoric Lepidophyte from the Carboniferous (With Plates IX–XI) — Harvey Bassler — 73

Intersexes in Plantago lanceolata (With Plates XII, XIII) — A. B. Stout — 109

Sexuality in Cunninghamella — Owen F. Burger — 134

Briefer Articles

Errors in Double Nomenclature — J. C. Arthur — 147

Current Literature

Notes for Students — 149

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London and Edinburgh*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Vo ume LXVIII Number 2

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued August 15, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, £1 12*s.* 6*d.* each; single copies, including postage, 3*s.* 6*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 18 Peking Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

VOLUME LXVIII NUMBER 2

THE
BOTANICAL GAZETTE

AUGUST 1919

A SPORANGIOPHORIC LEPIDOPHYTE FROM THE CARBONIFEROUS

HARVEY BASSLER

(WITH PLATES IX–XI)

The plant impressions which form the subject of this paper have in part been known for several decades, but the discovery by the writer of the key to their important and very interesting nature came as a pleasant incident in monographic studies of the very large and varied flora of the Maryland Carboniferous, and it is believed that the testimony which they contribute to a discussion of the problem of the phylogeny of the vascular cryptogams will justify the publication of this paper in advance of the large systematic work upon which the writer is engaged.

These plant impressions were long regarded as the sporophylls of *Lepidostrobus* (*Lepidophyllum*), and latterly (16) certain of them were thought to be the microsporophylls of some yet unknown type of *Lepidostrobus* or *Lepidocarpon* whose sporangia by their complicated form, not then understood, differed from those of the known species of *Lepidophyllum*, but the writer will show that they differ so fundamentally from all known cryptogamic sporophylls that it will be necessary to establish for their reception a new genus. The most distinctive feature of this group is a large lamellar sporangiophore developed in the radial plane of the strobilus from the superior (ventral) face of the sporophyll pedicel, bearing two large elongate sporangia, one upon each side, pannier-like; and it is this character which has suggested the name *Cantheliophorus*,

from *καν*θήλια, the classic term for packsaddles with baskets or panniers.

NATHORST in 1914 (16) had a vague suspicion concerning the nature of one of the species now included within the genus under discussion, *Lepidophyllum mirabile* Nath. from the Lower Carboniferous of Spitzbergen, and this he expressed when he ventured two hypotheses: (1) that we may be dealing with a sporangium in which longitudinal sterile plates such as we observe in certain sporangia of *Lepidostrobus* were developed to a point where they divided it into distinct and separate loculi, 2 loculi where the plate is simple and 3 where it forks, somewhat after the manner of the sterile plate figured by WILLIAMSON (37) (fig. 41); or (2) that this structure may consist of 2 concrescent sporophylls of which the lower bearing the blade is sterile, while the upper bearing 2 sporangia is fertile. While the nature of the material was such that its real character was not apparent, NATHORST'S very suggestive speculation concerning it prepares us somewhat for the discovery that the Carboniferous Lepidophytes are not the homogeneous stereotyped group they were long supposed to be.

The number of the sporangia subtended by each sporangiferous bract or sporophyll and the manner of their attachment have figured so largely in recent discussion of the phylogeny of the vascular cryptogams that we believe that some of this evidence should be re-examined in the light of the discovery of a truly sporangiophoric Lepidophyte in the Carboniferous, and after a comprehensive discussion of the characters of this new genus as a whole we shall devote some space to phyletic considerations.

Material

Lepidophyllum linearifolium Lesq., the first species of this group to be recognized, was described in 1880 by LESQUEREUX (13) from 5 good representative specimens from Pittston, Pennsylvania, and a fragmentary one, the nature of which is much in doubt, from Cannelton, Pennsylvania, all in the Lacoe Collection now at the United States National Museum,[1] and it is to be deplored that the

[1] Where, as in this case, there is slight discrepancy between the statement of occurrence on the labels pasted to the specimens and that made by LESQUEREUX in his *Coal Flora*, I shall record the former.

latter, consisting of nothing but a nondescript blade with the fragment of a pedicel attached, should have been selected for figuring. I am able to figure here the cotype from Pittston, together with several specimens from a large collection made by the writer in the Youghiogheny Gorge below Swallow Falls, Garrett County, Maryland, one of them showing a somewhat impoverished sporangium (partly cut away) still attached to the sporangiophore. Here, as in each of the other already recognized species of this group, the sporangiophore has been mistaken for a flattened sporangium. The second species to be recognized was noted very briefly by LESQUEREUX in 1880 in concluding remarks on *L. linearifolium*, and it was not until 1884 that there appeared in the *Coal Flora* (3:785) a brief specific description under the name *Lepidophyllum cultriforme*. Two "forms" were there recognized but were not separated even varietally. One from Cannelton is represented on *pl. 108, fig. 2*, by a misleading illustration of an interesting specimen showing 5 sporophylls attached in a series, and the other from Campbells Ledge near Pittston, Pennsylvania, is so inadequately represented on *pl. 107, figs. 13* and *14*, that the originals cannot be identified from the figures. Thus it came about that *Lepidophyllum cultriforme* in America became, not the name for a species, but for a group of cogeneric species characterized by little else than oblong "sporanges with their blades still attached to them," and any one of several species may actually occur at localities from which *L. cultriforme* is listed in the literature. This name will hereafter be restricted to the species from Cannelton, of which we are refiguring the specimen originally figured by LESQUEREUX and one other from the Lacoe Collection. The "form" from Campbells Ledge becomes a new species, *C. pugiatus;* fig. 27 is from a specimen from the Lacoe Collection.

POTONIÉ (**18**, p. 372) figured unsatisfactorily, without description, another species from Lower Silesia, under the name *Lepidophyllum waldenbergense*, and while NATHORST was engaged upon the study of the Spitzbergen collections, he secured from the Royal Prussian Bergakademie at Berlin the type material of the Silesian species in the expectation that it might help him to interpret certain structures in *L. mirabile*. In this he was disappointed,

but he redrew the types of POTONIÉ and reproduced them (**16**, p. 64, *fig. 16*), remarking that an apparently non-septate sporangium, in place, with a thin plate of tissue extending for a short distance beyond it, above, might indicate an intimate relation with *Lepidocarpon* if this plate of tissue proved to be part of an investing membrane about a megasporangium. The same paper (p. 62) describes another Spitzbergen species, *L. riparium*, which is compared with *Lepidocarpon*. Although the originals of *figs. 17* and *18* on *pl. 13* occur in juxtaposition, we shall not hereafter include in this species the first named. In his contribution to the Carboniferous Flora of Northeastern Greenland, NATHORST (**15**) has appended to a series of figures of "*Lepidophyllum* cf. *lanceolatum*" two figures (*34* and *35*), concerning which he remarks that it is difficult to ascertain whether or not they belong to the same species as the others. It seems apparent that they do not, but instead constitute a species of *Cantheliophorus*. We shall not attempt, however, the description of a new species from these figures.

To the several species already discussed, 5 sharply characterized new species (*novaculatus*, *ensifer*, *sicatus*, *robustus*, *grandis*) are added, based upon material collected by the author from Maryland, a sixth (*subulatus*) based upon material from Kansas in the Lacoe Collection at the United States National Museum,[2] and a seventh (*pugiatus*), already mentioned, based upon material from the Northern Anthracite Coal Basin of Pennsylvania.

Generic characters

In this genus the sporophylls, of which the organization is somewhat complex, are borne in spiral arrangement upon an axis in the formation of a strobilus. This axis has not been observed, but the nature of the cortex is apparently such that upon the disintegration or disruption of the cone a narrow linear fragment invariably remains attached to the proximal extremity of the sporophyll pedicel. The dismemberment of the cone at maturity was apparently very complete, for in a large collection of these forms only 3 cases were observed where sporophylls are

[2] This is the material listed by DAVID WHITE as *Lepidostrobus cultriformis* in Bull. 211, U.S. Geol. Survey, 1903, p. 105 (excl. Lacoe Coll. nos. 16092, 16093).

attached among themselves (2 of them are figured), and curiously enough in none of these is the axis itself preserved. This sliver-like fragment of the cone axis, for facility in subsequent discussion, we shall call the "foot," that part of it which extends upward from the point of insertion shall be known as the "toe," and the downward extension the "heel." The length of the toe is usually greater than the width of the sporangiophore, but in one American species (*ensifer*) it is characteristically very long and slender, and it is a fortunate circumstance that our best series of attached sporophylls belongs to this species, for it shows conclusively that units with such length of foot must be arranged spirally to be disposed as we find them in this series.

The sporophyll pedicel is usually nearly or quite normal or perpendicular to the axis of the cone, and as a rule it is straight, although in several species (*mirabilis*, *waldenbergensis*) it regularly curves slightly upward toward the distal extremity. To the inferior, dorsal, or abaxial face of the pedicel there is attached a plate of tissue which we shall call the "keel." This structure in some species (*robustus*, *cultriformis*, *subulatus*) is relatively wide and was probably membranous or scariose, maintaining its width from the heel to which it is attached, to the distal extremity of the pedicel where it terminates abruptly. In other species (*linearifolius*, *mirabilis*, *grandis*) it is developed only distad into a sort of semispatulate structure, which was sometimes rather stout and probably ligneous from development of sclerenchyma (*linearifolius*, *grandis*). To the extremity of the pedicel is attached the lamina or blade of the sporophyll. This varies in length among the species of this genus from 1 to 12 cm. It may be narrowly and sharply triangular in shape (*cultriformis*, *ensifer*, *sicatus*), 8 mm. or less in width at base, or slender and long-linear in shape (*linearifolius*, *grandis*, *novaculatus*) and only 2–3 mm. wide at base.

The single midrib is continuous with the pedicel at the bayonet-like attachment of the blade, but at this point the blade is often flexed sharply forward, and within certain limits the angle which the blade makes with the pedicel (we shall call it the "angle of inflexion") is characteristic of a given species. In certain species (*novaculatus*, *robustus*, *waldenbergensis*) this angle is nearly or quite

90°, which means that the blades were closely appressed and the cone very slender in consequence, while in other species (*linearifolius*, *grandis*) this angle is nearly or quite 180°, which is to say that the blade is in practical alignment with the pedicel, with a result that we have a very loose bristling cone, in one species (*linearifolius*) as much as 10 inches in diameter, a striking object! In certain species, particularly those with triangular blades, the blade for nearly or quite its entire length may be folded sharply backward, lengthwise along two parallel lines where the lateral stomatiferous furrows have weakened the lamina. These two furrows upon the underside of the blade are usually separated by the space of a millimeter or less, and the angle which the lateral portions of the blade make with one another ranges from 0°, where they are parallel, to 180°, where the blade is not at all reflexed or reduplicate lengthwise. In some cases (this is particularly noticeable in *linearifolius*) these two lines share the plication unequally, one of them sometimes indeed taking all of it. In some of the species (*sicatus*, *pugiatus*) the blade is folded back only throughout the proximal half, beyond which it flattens out or becomes actually concave above. Sometimes a faint longitudinal line may be seen upon each half of the blade, roughly midway between the stomatiferous furrow and the lateral margin. In one species (*novaculatus*), in which the blade is reduplicate only at the very base, we have noted with much interest a slight torsion which is always dextrorse in passing from the base outward, this without a single exception among over 100 examples observed. At the base the blades in some species (*ensifer*, *cultriformis*, *sicatus*) are abruptly cut across, with the lateral basal angles either sharply rectangular or rounded subangular, while in other species (*novaculatus*) the lateral basal portions of the blades are prolonged downward for 1–2 mm. into auricular processes.

Borne upon the superior, ventral, or adaxial face of the pedicel, in the angle made by the pedicel and the toe, is a large platelike structure, the sporangiophore, somewhat oblong-elliptical in shape; in length only slightly in excess of that of the pedicel, bearing 2 large elongate sporangia, one upon each side of it, attached at a point well forward, a short distance above the junction of the

pedicel and the blade. The surface may be nearly plane or it may be thrown into several low longitudinal folds. It is sometimes nearly smooth (*ensifer*, *sicatus*), but it is usually granulose to the unaided eye and minutely rugulose-bullate under the lens. In some forms the units of this sculpture are isodiametric, while in other forms some of them at least are distinctly elongate transversely, and sometimes the surface is very clearly transversely rugose even to the unaided eye (*riparius*, *subulatus*). Usually there is a line, a narrow bar or a low narrow longitudinal ridge, which we shall call the "brace," dividing the face of the sporangiophore into 2 regions, which will be referred to hereafter as the "inner" or "axillary field" and the "outer field." The outermost portion of the latter, particularly when it is differentiated, will be called the "crest." In species with a great development of crest (*cultriformis*, *subulatus*) this structure extended well upward between the sporangia of the superjacent sporophylls. In a cone in which the sporophylls are arranged spirally, a given sporophyll does not regularly occur exactly beneath the interval between the two next above it, and by reason of this fact the crest of the first mentioned sporophyll is usually slightly deflected in its course into this interval, and this deflection can often still be seen upon the crests of detached examples. From a point on the toe near the middle of the narrow proximal end of the sporangiophore the brace extends forward sometimes straight toward the point of inflection, while at other times it is parallel or nearly so with the pedicel throughout much or all of its length. In the first case the axillary field has the shape of a narrow isosceles triangle, with the base along the axis of the cone; while in the second case its shape is roughly oblong-rectangular, with the distal extremity more or less acute where the brace toward this extremity is deflected toward the pedicel, which may or may not be flexed upward slightly to meet it. The outer field is sometimes oblong-rectangular, but is usually somewhat arcuate, particularly distad. The outer margin of the crest is convex. The granulations upon the face of the sporangiophore may or may not extend across the surface of the brace. These granulations sometimes extend to the outer margin of the crest (*novaculatus*), while at other times they are limited strictly to

a definite linear-oblong area above the brace and close to it (*mirabilis*).

In a plane perpendicular to that of the sporangiophore at its distal extremity is a narrow plate, as much as 2 mm. wide, which will be called the "guard," for in certain species (*linearifolius*, *grandis*) its development is such that it serves obviously for the protection of the sporangia. This guard assumes a characteristic attitude for a given species, which may be expressed in terms of the angle which it makes with the pedicel, measured in the plane of the sporangiophore. This angle ranges from practically 90° to 150°. The sporangiophore with the upper termination of the guard often forms a distinct angular or cornicular process which will be called the "beak." The brace appears to terminate against the guard usually near the junction of pedicel and blade.

The oblong or elliptical sporangia are flattened against the sporangiophore and are attached to it apparently by a relatively narrow neck. In some species they appear to have been nearly coextensive, but usually the sporangia do not extend quite to the foot nor to the outer edge of the crest. The limited space between sporophylls at the axis of the cone would allow only for greatly flattened extensions of the sporangium, and this space appears not usually to have been invaded at all. The sporangia of *cultriformis* usually did not extend within 2.5 mm. of the cone axis. Although throughout this genus normal sporangia at maturity appear generally to have become detached from the sporangiophore, the writer has found several specimens with apparently normal sporangia still in place, from which it has been possible to cut away a part and reveal the sporangiophore beneath. Several of these are figured. What may have been defective or somewhat impoverished sporangia still attached to the sporangiophore are not really uncommon. The area upon the sporangiophore once covered by a sporangium which has become detached may sometimes be known from a faint impression of the latter, and indeed the shape and size of the sporangia in some cases (*cultriformis*, *subulatus*) are as yet known only from these impressions. The surface of the sporangium, which is often traversed by a distinct median longitudinal furrow, may be smooth, farinose, or fine-granular. These sporangia

may eventually prove to be microsporangia, but until undoubted megasporangia have been discovered we prefer to consider this genus homosporous. Recognizing that practically all known paleozoic Lepidophytes are heterosporous, these must have been preceded in the phylogeny by homosporous forms.

There appears to be no evidence of a ligule, but it must be recognized that negative evidence concerning an organ as delicate as this, in the case of carbonized impressions such as these under discussion, can have very little value.

Relation of structure to environment

The distal attachment of the sporangium by a relatively narrow neck has been looked upon rather generally as archaic or primitive and subject to two obvious disadvantages (33): (1) such a restricted channel for the passage of food and water to the developing spores may lead to nutritional difficulties as the sporangia increase in size; (2) it is mechanically weak.

The first of these ideas seems to have developed largely with that theory of descent among the Lycopods which holds that *Lepidostrobus*, with the linear attachment of its sporangium, is the more highly organized and modern, and *Bothrostrobus*, with the necklike attachment of its sporangium, the more primitive, for it furnishes one plausible explanation for this modification in descent. The trabeculae and sterile plates which extend upward among the spores in the large sporangia of some of the arborescent Lycopods would add confirmation to this view if they were developed, as BOWER (5) and others believe, in response to a demand for yet greater improvement in the mechanism of nutrition. Whether or not the restricted attachment of the sporangium of *Cantheliophorus* placed it at a disadvantage in competition with other forms may for the moment be left open. That this attachment is mechanically weak is obvious, but with proper compensation in protecting structures during the period of the development of the sporangium, this very weakness, instead of a disadvantage to the plant, may become a real advantage. In this genus, owing to the weakness of this attachment, the dissemination of the sporangia with their spores must have been very thorough, for the occurrence

of a normal sporangium in place upon a detached sporophyll is rare, while, on the other hand, the sporangia of *Lepidostrobus* held so tenaciously to the sporophyll and the latter to the cone axis that both among petrifactions and impressions we find long series of apparently mature sporangia in place. It is conceivable that the very mechanical excellence of this attachment might thus prove the undoing of a type in close competition with another with no such restriction upon the dissemination of its spores. This mechanical weakness in *Cantheliophorus* is well compensated for in protecting structures at the time when otherwise it might prove fatal. The most conspicuous of these is the guard and the blade. The latter protects in one of two ways. In species like *C. novaculatus* where it is flexible it is closely appressed, and in this way forms a dense, overlapping, protecting vestiture for the entire cone; while in other species, well illustrated by *C. ensifer*, it stands out formidably, rigid and sharp-pointed, like a bayonet. The simple but very efficient expedient by which this rigidity is secured is interesting from a mechanical point of view. It is secured by backward folding along the stomatiferous furrows near the midrib until the moment of inertia about the horizontal neutral axis of the cross-section approaches that about the vertical neutral axis; terms which express resistance to bending in a cantilever channel, or angle-beam of equal legs such as this. The sporangium is further protected on one side perfectly by the sporangiophore and on the other, in some species at least partially, by the crest and keel of subjacent and superjacent sporophylls respectively. Very short, lateral, flangelike wings on the pedicel serve as rests for the sporangia. The large radial sporangiophores with their tenacious hold upon both pedicel and axis, further strengthened by the brace and the guard, probably gave to the cone as a whole great stability during the period of the development of the sporangia.

In strobiloid types the increase in the volume of the sporangium by radial extension may be regarded as an efficient simple structural modification for increasing spore production, because this result may thus be accomplished without at the same time disturbing the cone by the multiplication of parts or by the modification and the increase in the size of the necessary protective structures.

Sporangia of *C. linearifolius* attain a length of 18 mm., rivaling those of *Lepidostrobus Rouvillei* Saporta and Renault, *L. Bertrandi* Zalessky, and *L. kentuckiensis* Scott, which range in extreme length to 16 or 17 mm. It would seem probable, therefore, that we are dealing with a relatively efficient type of cone.

Systematic position of genus

In the discussion of the relation of this genus to others of the Paleozoic we shall use the terminology of the systematic arrangement of the vascular cryptogams proposed in 1915 by BERRY (3, 4), and it may be prudent to give here very briefly, without annotation, the part of this classification which will concern us presently. The phylum Pteridophyta no longer embraces all the vascular cryptogams, but is restricted so as to include only the Filicales of the old scheme. The phyla Lepidophyta and Arthrophyta are established to receive the scale-leaved and the joint-stemmed vascular cryptogams respectively, thus:

PHYLUM LEPIDOPHYTA

Lycopodiales	
Lycopodiaceae	Lycopodiales of literature (referred to in this paper as the Lycopods)
Selaginellaceae	
Lepidodendrales	
Bothrodendraceae	
Lepidodendraceae	
Sigillariaceae	
Isoetales	
Psilotales	

PHYLUM ARTHROPHYTA

Class Sphenophyllae	
Sphenophyllales	
Class Calamariae	Equisetales of literature (excepting a few of the more recent contributions)
Equisetales	
Calamariales	
Pseudoborniales	
Protocalamariales	

In *Cantheliophorus* the sporophylls are arranged spirally upon the axis of the cone, and the slender, simple, uninervate sporophyll laminae show upon the under surface 2 lateral longitudinal grooves like the characteristic stomatiferous grooves of the usual paleozoic Lycopod leaf. Upon such evidence we must place this genus among the Lepidophyta, and we may have further evidence in

justification of this in the intimate and constant association of one species (*C. novaculatus*) with a species of "*Lepidodendron*" with small square or rhombic, closely packed bolsters, which we have suspected of being a part of the same plant. At a horizon two-thirds of a mile east of Westernport, Maryland (190 ft. below the horizon of the Davis seam), *C. novaculatus* is replaced by *C. robustus*, a very closely related species, and it may be significant in this connection that the small-bolstered species of *Lepidodendron* just mentioned is here replaced by a very similar one.

The only groups in the phylum Lepidophyta as it stands today are the Lycopods and the Psilotales. The latter constitute a small recent group of disputed position, believed by BOWER (6), THOMAS (32), and SCOTT (28) to be related more closely to the Sphenophyllales than to the Lycopods. They are characterized by lepidophytic leaf habit and furcate sporophylls supporting bilocular or trilocular synangia upon the adaxial surface. Concerning the former BOWER has said: "There is perhaps no character which marks off the plants of lycopodinous affinity from others so clearly as the constancy of the solitary sporangium . . . . it stamps this series of Pteridophytes as peculiar from all others"; to which may be added a statement from SCOTT (27): "The Lycopods constitute a wonderfully homogeneous group so neatly rounded off as to give little hold for any hypothetical link with other classes of plants."

The number and attachment of the sporangia and the position and the nature of the sporangiophore, when present, are matters of prime importance in the present classification of the vascular cryptogams, and at once the impracticability of placing *Cantheliophorus* with its conspicuous bisporangic sporangiophore among the Lycopods becomes evident. The objections to placing it among the Psilotales are almost equally insuperable, and there remains no alternative but to establish for its reception a new order, CANTHELIOPHORALES of the phylum Lepidophyta.

Phyletic relations

The question whether this group is either more or less primitive than the others of the phylum now arises for consideration. There has been a growing disposition in late years to regard the simple

relation of the Lycopod sporangium to its sporophyll as the result of reduction from more elaborate and intricate forms, but the evidence in support of this view has not been conclusive, although there appears to be very little against it. Although BOWER (7) believes that there is a preponderance of evidence in favor of amplification from simpler forms in the phyletic development of many of the Lycopods, he prefers to leave the matter "for the present open"; and Lady ISABEL BROWNE (10), after a brief impartial discussion of the question, likewise leaves it so. SCOTT (27) is inclined favorably to the alternative view, but he states: "Whether the simple relation between the sporangium and the sporophyll, which characterizes the Lycopod series, is native or acquired may be left an open question. The analogy of the Psilotales rather suggests the latter alternative, and all comparative morphology teaches how often progress consists in simplification." In 1908 Miss BENSON (2) made a very interesting contribution to this subject, in which she says: "It has been again and again suggested that we have in the lycopodinous 'sporange' a reduced structure which is homologous with the sporangiophore of the Sphenophyllales, but the evidence in favor of this 'reduction hypothesis' is still very inadequate."

SCOTT (28) reminds us that "the ventral pad of *Spencerites* has been compared to the sporangiophore and the sterile tissue in *Mazocarpon* also by Miss BENSON," but he concludes that "these suggestions are interesting but at present too hypothetical for any conclusion as to affinity to be based on them." It is the isolation of the Lycopods that has made phyletic hypotheses concerning them so inconclusive. In spite of great structural diversity, the Psilotales do after a fashion bridge the great gap between the Lycopods and the other Paleozoic groups, but the peculiar specialization (aberrancy) of this group reduces its value for comparative purposes, and in any event, as SCOTT has said, "it is not wise to rely much on evidence from a recent family in questions of remote ancestry." A distinct new Paleozoic group showing definite affinity with the Lycopods might then well be expected to contribute to this difficult problem facts of interest and importance. "Both fusion and septation have occurred in various instances,

and in any given case the proper initial attitude is to hold that either mode of origin may have been the source of the synangial state as it now appears" (BOWER 7). Let us seek out first the facts which might appear to harmonize with the "reduction theory." If this hypothesis is the correct one for the Lepidophytes, *Cantheliophorus*, with its elaborate bisporangic sporangiophore expanded platelike in the median vertical plane of the sporophyll, would be looked upon as the representative of some primitive ancestral type, from which by reduction and simplification the Lycopods, with a solitary median sporangium resting directly upon the pedicel of the sporophyll, might have resulted. Are there any structural details in *Cantheliophorus* which are generally conceded to be primitive? The distal attachment of the sporangia is often considered to be such, but this opinion has developed so largely out of certain theories of descent, themselves resting often upon very insecure foundations, that while it should not be forgotten in summing up the evidence, it can hardly be thought of as having much weight. Assuming the correctness of this hypothesis, we might expect to find in the more reduced of several groups vestiges of structures which are functional or at least more extensively developed in ancestral forms or in the less reduced descendants of these ancestral forms. I have already quoted the suggestion by SCOTT that the ventral sporange-bearing prominence upon the pedicel of *Spencerites* may be the vestige of a sporangiophore. Miss BENSON figured and discussed at some length *Mazocarpon* from the Upper Carboniferous, with its great central core of sterile tissue and two lateral "sporogenous regions," and in the same paper also a new heterosporous form, *Lepidostrobus mazocarpon*, from the Lower Carboniferous of Burntisland, Scotland, likewise with a great central mass of sterile tissue, both in the microsporangia and megasporangia, with the sporogenous tissue arranged in an arc about it above, and she states that "it would be a very natural sequence that the sporogenous region of a single sporangiophore should become confluent, and the gradual reduction of the sterile tissue to a mere 'archesporial pad' and pedicel would next follow."

In 1872 WILLIAMSON (37) described and figured a *Lepidostrobus* (*L. Veltheimianus* Scott) in which a single continuous plate of sterile tissue usually in a median position arises from the slightly projecting subarchesporial pad and extends upward into the sporangial cavity. WILLIAMSON believed it to be "coextensive with the entire length of the sporangium," but BOWER (5) figures a section in which it does not extend quite to the distal extremity. In 1914 Mrs. AGNES ARBER (1) demonstrated the presence in *Lepidostrobus Oldhamius* Willm. of a similar plate of sterile tissue which also "died out toward the distal end of the spore sac," and the same structure was observed by this author in *L. foliaceous* Maslen, *L. Binneanus* Arber, and in an unnamed species from the Coal Measures of Great Britain discussed and figured by BOWER in 1894. BOWER also called attention to the irregular trabecular processes that "spring upwards from the floor of the sporangium (of *Lepidostrobus Brownii*) and project a considerable distance into the cavity. They are not scattered indiscriminately over the floor of the sporangium, but arise from a projecting ridge." RENAULT (21) shows similar structures in the sporangium of *L. Rouvillei* Sap. and Ren. from the south of France. Accordingly Mrs. ARBER concludes that "some form of sterile upgrowth from the sporangial floor may eventually prove to be characteristic of all forms of *Lepidostrobus* which are homosporous or microsporous, such a structure not yet having been observed in megasporangia."

The median sterile plate in the sporangium of a number of species of *Lepidostrobus* is singularly suggestive of that median plate in *Cantheliophorus* which constitutes the sporangiophore, but even should we accept their homology, and this we may not so easily escape, this fact, if considered without reference to other facts, could as well be adduced in support of one as of the other of the opposing phyletic theories of reduction and amplification. Are there any facts which might suggest to us the course of the events in the descent of these groups? Facts from ontogeny are of the utmost importance in the solution of phyletic problems, but from the very nature of fossils such facts are not usually available. We are fortunate, however, in having a few facts concerning the

immature sporangia of *Lepidostrobus*. In the immature sporangia near the apex of the cone of *L. Brownii* (Unger) Schimper, BOWER (5) has observed that "the bands of sterile tissue extend to the upper wall of the sporangium," but he has been "unable to establish beyond doubt the fact of the tissue connection between them and the wall." A section through a cone of *L. Oldhamius* Williamson, "passing obliquely through the apex and displaying the internal structure of the immature sporangia with great clearness," has been figured and discussed by Mrs. ARBER, as follows (see fig. 42):

> The sterile plates in these young spore sacs are massive and well preserved and give rise with great regularity to two smaller lateral processes one on either side. The branches are not directly opposite to one another and the main process is continued above its branches for a considerable distance. That these outgrowths are of the nature of plates running in the direction of the long axis of the sporangium and are not merely peglike structures is evidenced by the fact that they present a general similarity of appearance in the seven sporangia in which they are visible in the section presented in *pl. 21, fig. 1*. . . . . Another point which is established by comparison of the different sporangia is that the sterile plate died out towards the distal end of the spore sac. . . . . In older sporangia the sterile plates are relatively less important; the lateral branch plates seem to shrivel and disappear quite early.

These very interesting facts seem susceptible of an interpretation favorable to the reduction theory, for if there is a phylogenetic recapitulation in the development of this sporangium we can scarcely escape the conclusion that at one time in the history of the race the sporangium was divided more or less completely by a stout septum into 2 loculi, and perhaps at an earlier period still into 4 loculi if we may be permitted to interpret the branches of the sterile plate of the more immature sporangia as the vestiges of a transverse sporangial septum in some ancestral form; for if we observe the relatively great size and development of this sterile plate with its branches in the least mature of this short series of sporangia, it will at once seem highly probable that in sporangia just a little less mature these branches would extend quite to the lateral wall. For the first of these hypothetical ancestral forms one could not wish for a representative more satisfactory than *Cantheliophorus*, and for the second more remote tetrasporangic

ancestor the Calamariales suggest themselves. Is there further evidence to show that the former is near such a line of descent? Unfortunately we have here no structural material which might show us stages in the development of the sporangium, but in mature sporangia of our collections the distinct median longitudinal furrow regularly encountered in several of the species may have significance in this connection, and the differentiation of the face of the sporangiophore into two more or less distinct fields might have some bearing on this matter, and it is perhaps not a mere coincidence that these fields are most sharply marked in one of the oldest known species, *C. mirabilis* from the Lower Carboniferous. While such evidence for a tetrasporangic ancestor for *Cantheliophorus* and through it for the Lycopods is exceedingly weak, it cannot be ignored.

In *Cantheliophorus* the sporangiophore is dorsiventral, while the symmetry of this structure in the most abundant of the Calamariales, *Palaeostachya* and *Calamostachys*, with a few exceptions is radial, and unless we can indicate a form somewhat intermediate between types of such essential difference, theories involving their affinity must remain inconclusive. *Calamostachys* (*Arthropitystachys*) *Grand'Euryi* Renault and *Calamostachys* (*Arthropitystachys*) *Decaisnei* Renault (**19, 21**), two species based upon petrified material from Saint-Étienne, appear to constitute such an intermediate type, for in each there is a stout plate of sterile tissue singularly like the sporangiophore of *Cantheliophorus*, which spans more or less completely the space between the sporangiophore and the bracts of the whorl above, interposing a vertical wall between sporangia borne distad upon the same sporangiophore. There is slight development also of similar tissue in this plane beneath the sporangiophore (fig. 33). Even though it be admitted that these 2 species may possibly lie near the line of descent, it may be objected that there is still a great fundamental difference between them and *Cantheliophorus*, inasmuch as the arrangement of the sporophylls is verticallate in one case, while it is spiral in the other. Such an objection, however, might easily be dismissed with a reference to *Spencerites*, a form with verticillate sporophylls, which has been placed without question in the exclusive group of the Lycopods.

Will the comparison of stem anatomy throw any favorable light upon the question of a possible remote phyletic connection between the Calamites and the Lycopods? A comparison of the stem structure of such lycopodian forms as *Sigillaria Menardi* (BRONGNIART 9) (fig. 13) with a calamitean form such as *Calamites* (*Asteromyelon*) *Augustodunense* (RENAULT 23) (fig. 14), an appendage of the stem, probably a root, of some species of *Calamites*, will at once reveal a similarity which extends to details. In both the xylem consists of a ring of somewhat wedge-shaped elements, distinct from one another but nearly or quite in contact in their wider portions except for narrow interposing medullary rays. Each xylem "wedge" consists of both primary and secondary xylem, the relatively small bundle of centripetal protoxylem occupying the apex. In each case the outer side of the protoxylem bundle is occupied by small spiral tracheids, while the remainder of the bundle, the convex inner portion, consists of larger scalariform tracheids. These primary strands are distinctly separated from one another. The secondary xylem consists of large radially arranged scalariform tracheids. Beyond the xylem is a narrow zone of phloem, and beyond this the cortex. Agreement such as this in the stem anatomy of forms from different phyla is very impressive.[3] On the other hand, we can think of no lycopodian stem which even remotely suggests the characteristic triarch or hexarch protostele of the Sphenophyllales. The evidence from anatomy then gives a certain plausibility to the suggestion that *Calamostachys Grand'Euryi* Ren. and *C. Decaisnei* Ren. may represent a calamitean ancestor of *Cantheliophorus*. "True phylogenetic homologies may fairly be expected to be traced between plants which appear to belong to the same natural series" (BOWER 5), and accordingly the brace in the sporangiophore of *Cantheliophorus* and the radially symmetrical sporangiophore in *Palaeostachya* and *Calamostachys* may come to be considered

[3] If by chance anyone should contend, though it is rather beside the point, that the force of this comparison is weakened somewhat by the fact that centripetal xylem, as VAN TIEGHEM first pointed out, is common to the roots of all vascular plants, we should say that the force of this comparison is correspondingly reinforced by the fact that this type of protoxylem characterizes the stem of *Protocalamites pettycurensis* (Scott) Lotsy (SCOTT 28) from the Lower Carboniferous of Scotland.

homologous. The outer field of the sporangiophore of *Cantheliophorus* might then be represented in the 2 species of *Calamostachys* just considered by the plate of sterile tissue above the sporangiophore, and the guard in one case, which is peltate with respect to the brace considered alone, would then become the homologue of the peltate extremity of the sporangiophore in the other.

The sporangiophore of *Calamostachys Decaisnei* Ren. which we have reproduced is inclined slightly toward the bracts beneath it. This may be accidental, but with just a little more inclination, together with the closing of the space between it and the bracts below by a sterile plate (of which we already see some development), we should have a sporangiophore almost precisely like that of *Cantheliophorus*. With coalescence, further, of the pair of sporangia upon each side of it and the concrescence of the 2 subjacent bracts, we should have to all outward appearances *Cantheliophorus* itself. Whether or not there are two parallel vascular bundles in the midrib of the blade of *Cantheliophorus* as possible evidence of such a history we have at this time no means of knowing, but we may recall with some interest in this connection that a species of *Sigillaria* from Autun does bear a leaf with 2 vascular bundles in the midrib, and it seems significant that in general the stem anatomy of this species closely resembles that of *S. Menardi*, which of all the known Lycopods is perhaps closest to the Calamites in this respect, as I have shown elsewhere in this paper, and according to the theory here set forth one of the most primitive. It was RENAULT (20) in 1879 who described this species under the name of *Sigillariopsis Decaisnei*.

It may be well in passing to remind our readers that according to the theory here proposed the sterile plates within the sporangia of *Lepidostrobus* were not developed, as BOWER suggested, primarily to serve for the nourishment of the spores, but it is probable that they did well serve that purpose in a sort of secondary or incidental manner, transferring nourishment to the interior of the sporogenous mass in the early stages of its development, and finally wasting as they yielded their own substance to the developing spores.

There is yet one weakness (8) in this theory of the descent of the Lycopods. "It is not enough to suggest reduction on mere

grounds of comparative convenience; to make the suggestion convincing in any group where general reduction is believed to have occurred it will be necessary to prove that the sum of nutrition, from whatever source, has diminished in the course of descent and that the reduced spore output has been the result. Until this has been shown to have occurred in any case, there seems no sufficient reason to accept as more than a quite open hypothesis any suggestion of general reduction of the sporophyte" (BOWER 7). "The hypothesis of relative primitiveness has then logically prior claim and must be accepted as a working theory until good grounds can be given for preferring that of reduction" (TANSLEY 31).

With the development of a dendritic habit among certain groups of the Lycopods came greater capacity for spore production, and this was followed up structurally in several ways, for the multiplication of spores in a homosporous plant, with equal chances of dissemination, must be considered an advantage as long as these spores can be properly matured, and in many forms it was probably not merely an advantage but a necessity to produce spores to the limit of their capacity in order to maintain themselves in the struggle for existence. Unfavorable environmental conditions, such as slow unfavorable climatic changes, may be expected to decrease the capacity for spore production, and this among plants that have been producing spores to the limit of their capacity will inevitably lead to one or more of several possible forms of reduction. There may be reduction in the number or the size of the sporangia or sterilization of some of the potential sporogenous tissue or archesporium.

During geological time there have been great periods of marine transgression, punctuated by periods of regression or continental emergence, usually accompanied by orogenic disturbances, and each of these great cycles of earth experience constitutes a geological period. Changes in the distribution and altitude of land masses toward the close of each of these periods were attended by climatic changes which were sometimes profound. They usually tended somewhat toward aridity, accompanied as a rule by a reduction of the mean annual temperature. To geologists the evidence supporting such facts is today so eminently satisfactory that they

have become almost axiomatic and may be accepted here without argument. The regular recurrence of red beds and sometimes salt and gypsum-bearing deposits toward the close of each great period conveniently marks these subperiods of continental expansion. In the Appalachian province where the Paleozoic record is unusually complete, we find the Juniata and Red Medina at or near the top of the Ordovician, the Salina toward the top of the Silurian, the Hampshire and Catskill occupying the same position in the Devonian, the Mauch Chunk in the Mississippian, and many red beds in the Permo-Pennsylvanian, the lowermost already occurring well toward the base of the Conemaugh. That periods of relative aridity and reduced temperature are in general unfavorable to plant life scarcely admits of argument, and thus during the descent of the cryptogams there have repeatedly been periods of stress during which some of them, accustomed to a certain habitat, were in all probability forced to adopt some program of reduction of the sporophyte in order to maintain themselves there. Allied forms, on the other hand, might be expected to occupy certain sheltered coastal regions where in spite of cold or general climatic aridity they could maintain themselves with little or no reductive changes, and thus it would come about upon the return of generally favorable conditions that the reduced and the primitive types might occur together. Arboreal types by reason of the specialization incident to this habit were doubtless upon the whole more sensitive to changes in the environment than the lowly herbaceous or suffrutescent forms, and it would seem very difficult to account for the *absence* of evidence of reduction among dendritic types that had survived the unfavorable periods toward the close of the Silurian, the Devonian, and the Mississippian.

Whether or not the Psilotales, the Isoetales, and the Lycopodiales (the last named, it must be remembered, are restricted in this paper to the Lycopodiaceae and the Selaginellaceae) belong to the phyletic line here proposed for the Cantheliophorales and the Lepidodendrales, is the question that now suggests itself. "When in a tissue tract the distinction between vegetative and sporogenous cells takes place late in the individual the presumption is that the distinction has been of late origin in the race. On this

basis the conclusion has been formed in certain cases that an increase in number of sporangia by septation has occurred. It is concluded that these late differentiated sterile tracts were once in the race fertile and that they were subsequently diverted from this previous condition; in fact that the ontogenetic development reflects the evolutionary history. This is exemplified in the synangia of *Tmesipteris* and the sporangia of *Isoetes*" (BOWER 7).

In the ontogenetic development of the sporangium of *Isoetes* and *Lycopodium* and the synangium of *Tmesipteris* (one of the 2 genera of the order Psilotales) there are no structures developed which might, like the sterile tissue within the immature sporangium of *Lepidostrobus Oldhamius*, suggest reduction in descent from a more intricate or elaborate form. The relation of the Isoetales to the remaining orders of the Lepidophytes is still very obscure, but is certainly closer to the Lepidodendrales than to any other, if we may judge by the radial elongation and insertion of the solitary sporangium; the trabeculae within this organ suggesting the rodlike or peglike processes within the sporangium of *Lepidostrobus Brownii;* the ligule; the secretory strands, comparable to parichnos observed in the leaves of one species; the great development of cortex, analogous to the enormous development of secondary cortical tissue, chiefly phelloderm, in the tree Lycopods of the Paleozoic; the secondary increase in the stem in which new zones of cells may have periodically taken up the cambial activities; and the dichotomous, monarch roots, like those of *Stigmaria*.

We are inclined to believe with BOWER, SCOTT, and THOMAS (32) that the furcate sporophyll with a synangium (not much unlike the group of sporangia upon the sporophyll of *Sphenophyllum majus*) resting upon its adaxial surface below the point of bifurcation in the Psilotales and the triarch stele with the xylem elements extending to the center in the smaller branches of *Psilotum* would seem to indicate that this group is related to the Sphenophyllales perhaps more closely than to any other. The stele in the stem of *Psilotum* is not much unlike that in the axis of the cone of *Cheirostrobus*, one of the Sphenophyllae from the Calciferous sandstone of Pettycur, Firth of Forth; and this, with the formation of secondary xylem at the base of the aërial stem and in adjoining parts of the rhizome

in old plants, "materially strengthens the anatomical analogy" (Scott 28). Concerning the relationship of the last remaining order of the Lepidophytes Scott states: "Recent discoveries appear to show conclusively that *Selaginella* had no direct connection with the Lepidodendrae, but sprang from a distinct and equally ancient herbaceous stock. No light has yet been thrown on the ancestry of *Lycopodium*, which certainly had no near relation to any Paleozoic forms in which the nature of the spores has been determined." The facts presented in this paper in no wise call for a revision of these conclusions. Already in the Paleozoic there were forms of heterosporous Lycopods agreeing closely with *Selaginella* of the present day, and although there has been specialization along different lines during descent, the lowly habit of the members of this group appears to have kept them so much out of competition with the more aggressive arborescent forms that there has been in all probability but little amplification or reduction throughout this great lapse of time, and together with *Lycopodium* they may perhaps be looked upon as something of a "persistent primitive type" (Huxley).

In 1909 Lady Isabel Browne estimated the prevailing theories of Lycopodian descent as follows:

> The weakest part of the theory that the Lycopod sporangium is the result of coalescence and fusion of free sporangia lies in the fact that it is among the heterosporous forms (*Lepidostrobus Mazocarpon*, *Mazocarpon*, *Isoetes*), presumably less primitive than the homosporous types, that what are regarded as the remains of a septum are most strongly developed. On the whole, the sterile tissue present in the above mentioned forms is much in excess of that found in *Spencerites*[4] or in most species of *Lycopodium*. Similarly, on Bower's hypothesis that the sporangia of the synangia of the homosporous Psilotaceae represent the loculi of a septate sporangium, it is curious that indications of intermediate stages in the process of septation should be more marked in several heterosporous than in any homosporous members of the Lycopodiales.

The significance of these difficulties regarding the prevailing theories of Lycopodian descent becomes apparent with the proposition that the Lycopodiales, the Lepidodendrales, and the Psilotales

[4] We are inclined to believe with Watson (33, p. 391) that *Spencerites*, although it may have certain archaic characters, in reality is not primitive and may not even be homosporous, for as yet we have had no means of knowing that the spores are not indeed microspores.

are related among themselves more remotely even than was long supposed, for the evidence now in hand appears to indicate that upon the whole the first of these is primitive, the second reduced from possible pro-Calamarian[5] ancestry, and the third, in the descent of which both amplification and reduction probably played minor successive rôles, specialized from pro-Sphenophyllaceous stock. The belief once prevalent that the Lycopods are related through the Psilotales to the Sphenophyllales and only remotely through the latter to the Calamariales will accordingly have to be abandoned.

Anatomical considerations

With the discovery of structural material of *Cantheliophorus* we may expect facts of great interest. If our interpretation of this form is correct, we may expect to find within the brace the fibrovascular bundle which supplies the sporangia. In the large sporangiophores of *C. linearifolius*, at the insertion of the brace upon the toe, there are minute striae which in passing from the proximal extremity of the brace into the toe, instead of bending downward as one might expect, toward the insertion of the pedicel, bend upward, and this may perhaps be interpreted to signify that here the brace at its insertion may already have been dragged slightly downward during the evolution of the race from Calamarian or pro-Calamarian stock, somewhat after the manner of the sporangiophore of *Paleostachya vera* Seward (12), and if such is the case then we should not be surprised to find that the vascular strand makes a slight loop forward in the toe above the insertion of the brace, comparable to that in the species of *Paleostachya* just cited. It would seem further to be quite in keeping with our theory of reduction for *Cantheliophorus* to find 2 parallel vascular strands in the midrib corresponding in a way with the 2 close parallel lines sometimes seen over the median nerve upon the adaxial surface of the blade, but such suggestions in the present state of our knowledge are perhaps unduly speculative.

[5] This is a hypothetical Calamarian ancestor, not necessarily proto-Calamarian.

Technical discussion of the species

There now follows a discussion of the species of this genus which will be technical and very brief, given in the interest primarily of stratigraphic correlation, for we believe that the members of a group with structure as intricate and diverse as this will have high stratigraphic value if treated with great systematic refinement. Details of structure already given will not as a rule be repeated here, and the discussion will be further condensed by a tabulation of the considerable number of dimensions involved.

The stratigraphy of the Pennsylvanian Period in western Maryland and adjacent parts of Pennsylvania and West Virginia has recently been studied critically by CHARLES K. SWARTZ, assisted by W. A. PRICE and the writer, among others, and a preliminary report on the results of these studies is about to be published by the Maryland Geological Survey,[6] but reference to the "horizon of the Davis seam," to the "top of the Pottsville formation," and to the "top of the Allegheny formation" in subsequent remarks, calls for a very brief statement of final conclusions reached with regard to the limits of the Allegheny formation, inasmuch as the standard section for this region has undergone important revision. In the Georges Creek basin the Ames or Crinoidal marine fauna occupies a position about 550 ft. below the base of the Pittsburgh seam; the Brush Creek marine fauna, a position 250 ft. below the Ames; and the Davis or "Split-six" coal (the topmost unit of the Allegheny formation), 115–120 ft. below the Brush Creek.[7] The thickness of the Conemaugh formation is thus a little over 900 ft., that of the Allegheny (upon stratigraphic and floral evidence) about 265 ft., and the Pottsville from the base of the Allegheny to the great unconformity at the top of the Mauch Chunk red shales between 200 and 250 ft.

CANTHELIOPHORUS LINEARIFOLIUS (Lesquereux).—The essential characters of this species are a very long slender blade perpendicular to the cone axis, a very large oblong sporangiophore with granulose

[6] A fuller discussion will be presented in the "Monograph of the Carboniferous of Maryland," which will be published at a later date by the State Survey.

[7] To the westward of the Georges Creek basin there is a gradual thinning of that part of the Conemaugh section above the Brush Creek marine horizon.

surface, and a well developed guard which is not, like that of *C. grandis*, of a length greater than the width of the sporangiophore. The brace is usually distinct.—Figs. 1, 2, 8–10.

Typical material has been collected from the roof shales of Coal B[8] at the Boston mine, Pittston, Luzerne County, Pennsylvania, and also from a locality high on the steep eastern slope of the gorge of the Youghiogheny River, directly south of the mouth of Deep Creek, 1 mile below Swallow Falls, Garrett County, Maryland, at a horizon in the Allegheny formation (near that of the Lower Kittanning coal) 170 ft. beneath the horizon of the Davis seam.

Cantheliophorus grandis, n.sp.—This species is well characterized by a long slender blade perpendicular to the cone axis, but apparently less rigid than that of *C. linearifolium*, and by a large sporangiophore with a very long guard which is nearly perpendicular to the pedicel. It terminates in an acute, abruptly upturned beak. The somewhat rugose-undulate surface appears, with the aid of a lens, to be minutely rugulose-bullate in part. The brace is not conspicuous.—Fig. 3.

The type material has come from a shale lens beneath the massive friable conglomeratic sandstone in the rock quarry south of the county road, at Holmes, West Virginia, on the Baltimore and Ohio Railroad, 1 mile west of Corinth, at a horizon very close to the top of the Pottsville formation.

CANTHELIOPHORUS CULTRIFORMIS (Lesquereux).—The conspicuous features of this species are a large wide sporangiophore and a wide keel which with the form and attitude of the blade serve well to distinguish it. The latter curves slightly upward beyond the middle and usually opens somewhat at the same time. The surface is granulose or, like the last, minutely rugulose-bullate; the units like those of the next species are somewhat transversely elongate.—Figs. 5–7.

This species occurs in the shales of the floor of the Darlington coal at Cannelton, Beaver County, Pennsylvania, correlated by WHITE (36) with the Upper Kittanning.

Cantheliophorus subulatus, n.sp.—This species is sharply defined by the expansive development of the sporangiophore and

[8] The more important coal seams in the anthracite field of Pennsylvania beginning at the base of the Lower Productive Measures (Allegheny formation) have been listed alphabetically in certain State Geological Survey publications. Coal B is supposed to be the equivalent of the Lower Kittanning of western Pennsylvania.

the keel, and by the straight, relatively short, slender blade projecting from the extremity of the pedicel without appreciable flexure, the lateral portions folded sharply back for practically their entire length. The surface of the sporangiophore is rugose with minute, close, irregular transverse folds which vary much in distinctness in different specimens.—Figs. 11 and 12.

The species occurs in the Cherokee shales (Kittanning group of the Allegheny formation) at the Penitentiary shaft, Lansing, Kansas, and in the floor shales of the Darlington coal (Upper Kittanning) at Cannelton, Pennsylvania (Lacoe collection no. 16100 U.S. Nat. Mus.).

Cantheliophorus ensifer, n.sp.—The salient features of this species are great length of toe (which distinguishes it at once from all others known to us at this time), a straight, rigid, narrowly triangular blade which makes a wide angle with the pedicel, and the smooth surface of the sporangiophore, which is usually thrown into several rather prominent longitudinal folds. The guard extends forward in a position directly above and usually in contact with the superior surface of the blade.—Figs. 15 and 16.

It is abundant at a horizon 1.25 miles below Swallow Falls, Garrett County, Maryland, along the old lumber tram on the steep eastern slope of the Youghiogheny River gorge, not far below the mouth of Deep Creek, about 400 ft. below the horizon of the Davis seam (the top of the Allegheny), and 130 ft. below the top of the Pottsville formation.

Cantheliophorus novaculatus, n.sp.—The noteworthy features of this species are the linear blade reduplicate only near the base, flexed forward into a position nearly parallel to that of the axis of the cone, and the sporangiophore with a fine granulose surface and a distinct brace. The keel was never wide, and it appears to have been rather frail, for often it is missing entirely.—Figs. 29 and 30.

This species is one of rather wide distribution in the Maryland area, and up to this time has been found only within the Allegheny formation. It has been collected by the writer near Warnocks Station, West Virginia, along the Western Maryland Railway, 25 ft. below the horizon of the Davis seam (top of the Allegheny); at Barrelville and Sunnyside from the roof shales of the Parker Coal, 50 ft. below horizon of Davis seam; south of Franklin, West Virginia, along the Western Maryland Railway, 75 ft. below the Davis; on the north fork of Jennings Run, northwest of Wellersburg, Pennsylvania, 95 ft. below the Davis; along foot path up the cliff above the Western Maryland

Railway west of Westernport, Maryland, 115 ft. below the Davis; along the Western Maryland Railway opposite Dodson, Maryland, 165 ft. below the Davis and along the Baltimore and Ohio Railroad; opposite Luke, Maryland, at the base of the Allegheny, 265 ft. below the horizon of the Davis. In the southern anthracite field of eastern Pennsylvania it has been collected at several localities from the roof shales of the Buck Mountain, or Twin Coal, 3 miles south of Tremont, Schuylkill County, Pennsylvania, in the Sharp Mountain Gap of Swatara Creek.[9]

Cantheliophorus robustus, n.sp.—This species is very similar to *C. novaculatus* in general appearance, but may be distinguished by greater relative width of the sporangiophore and of the keel, while the surface of the sporangiophore is smooth or farinose and not granulose. It is usually slightly larger throughout, but there is considerable range in size within a single collection.—Figs. 25 and 26.

The species occurs abundantly 1 mile east of Westernport at a horizon 190 ft. below that of the Davis seam.

Cantheliophorus sicatus, n.sp.—Among the more noteworthy features of this species is the smooth sporangiophore with surface usually thrown into several longitudinal folds. The blade is short, as a rule not much over 12 mm. in length, and makes an angle with the pedicel customarily of 150–180°, but the range must be given as 120–180°. It is narrowly triangular, usually sharply reduplicate and straight, but is sometimes curved well upward and may be open (nearly flattened) for most of its length.—Fig. 28.

In Maryland we made two large collections of this species from the Allegheny formation, one of them 1.5 miles east of Stoyer along the Western Maryland Railway associated with *C. linearifolius* (which see), 1 mile below Swallow Falls on east bluff of the gorge of the Youghiogheny River, Garrett County, 170 ft. below the horizon of the Davis seam. It occurs somewhat sparingly in the lowermost Allegheny, one-eighth of a mile east of Piedmont, West Virginia, in shales above the upper seam of coal exposed along the Baltimore and Ohio Railroad tracks, 260 ft. beneath the horizon of the Davis seam, and also in the shales above the Lower Coal exposed a short distance to the eastward of the last along the Baltimore and Ohio Railroad at a horizon 270 ft. beneath that of the Davis seam (possibly in the uppermost Pottsville). There is some question as to the precise systematic position of 2 imperfect specimens of *Cantheliophorus* very close to *C. sicatus* from a horizon east of Bond along the

[9] Listed as *Lepidophyllum cultriforme* by DAVID WHITE, 20th Ann. Rept. U.S. Geol. Survey, 1900, p. 825.

Baltimore and Ohio Railroad, 430 ft. beneath that of the Davis seam (165 ft. plus or minus beneath the top of the Pottsville), and while it is not prudent to extend the range of this species upon the testimony of questionable material, I shall make a record here awaiting further collection.

Cantheliophorus pugiatus, n.sp.—This species is not far different from the last, but as a rule it is larger, with a blade usually more than 15 mm. in length, making an angle with the pedicel ranging from 100° to 150°, but generally falling between 110° and 140°. The keel is wider. We have figured a typical specimen of each of these related species which will usually serve well to distinguish them, but we must caution that certain small atypic forms of this species might be confused with the last.—Fig. 27.

The type material in the Lacoe Collection at the United States National Museum has come from the Pottsville formation at Campbells Ledge near Pittston, Luzerne County, Pennsylvania.

CANTHELIOPHORUS WALDENBERGENSIS (Potonié).—This species has the general habit of *C. novaculatus* and *C. robustus*, but the keel is distinctly different, showing great expansion from the heel, where it is very narrow, to the distal extremity of the pedicel, where it terminates rather abruptly.—Figs. 19–21.

The type material has come from the Waldenberg series, equivalent in general to our Lower Pottsville from Segen-Gottes-Tiefbau near Altwasser, Lower Silesia.

CANTHELIOPHORUS RIPARIUS (Nathorst).—This species may be compared with *C. subulatus*, but the beak is more prominent, the sporangiophore relatively longer, and the keel narrower. The sculpture on the surface of the sporangiophore of this species, however, will alone serve to distinguish it.—Fig. 4.

The type was collected by *Norberg* in 1913 from the Lower Carboniferous at Örretelven, Spitzbergen.

CANTHELIOPHORUS MIRABILIS (Nathorst).—This species has the habit somewhat of *C. linearifolius* and *C. grandis*, but the resemblance to these species is not close. One of the most distinctive features is the sharp differentiation of the fields of the sporangiophore.—Figs. 22–24.

The material upon which the species has been based was collected in 1913 by the Hoel-Straxrud Expedition from the Lower Carboniferous or Culm at

TABLE I*

Cantheliophorus	Diameter of cone	Length of sporangiophore	Width of sporangiophore	Length of blade	Width of blade at base	Inflexion, blade to pedicel	Length of pedicel	Length of toe	Length of heel	Width of keel near heel	Width of keel near point of inflexion	Length of guard	Inclination, guard to pedicel	Length of sporangium	Width of sporangium	Formation
1. linearifolius (Lx.) .	250±	18–20	6–9	100±	2–3	135–190°	17–18	5–6	1–2	0–0.5	2–3	5	120–130°	15–18	4–6	Middle Allegheny (Kittanning Group), Pa., Md.
2. grandis. n.sp.....	200±	15±	9–12	90±	2	160–180°	11–12	6–7	1–2	0–0.5	2–2.5	10	100–110°	?	?	Uppermost Pottsville, W.Va., near Md.
3. cultriformis (Lx.) .	75±	15	7–10	20–30	3–4	150°	13–15	7	3	3	3	3–4	120°	10	5	Middle Allegheny (Kittanning Group), Pa.
4. subulatus, n.sp...	90±	14–17	12±	20–25	3	180°±	12–16	3–6	3–5	4–6	4–6	4±	120°	10	5	Middle Allegheny (Kittanning Group), Kan., Pa.
5. ensifer, n.sp.....	60–70	8–11	6–8	14–26	5–8	135–180°	6–8	10–14	2–4	1.5	1.5	3–4	120–130°	8	4	Upper Pottsville, Md.
6. sicatus, n.sp.....	30–40	5–7	2.5–3	9–15	4	120–180°	6	2	1–2	1	1	2–2.5	120–140°	5–7	1.5–3	Middle Allegheny to uppermost Pottsville, Pa., Md., W.Va.
7. pugiatus, n.sp....	30–50	6–10	3–4	15–25	4–5	100–150°	6–8	2–2.5	2–2.5	1–1.5	1–1.5	2–4	120°±	7	3	Upper Pottsville, Campbells Ledge, Pittston, Pa.
8. novaculatus, n.sp..	20–30	7–8	3–4.5	15–40†	1.5–2	90–100°	5–7	2.5–4.5	1–2	0.5–1.5	0.5–1.5	2–2.5	100–120°	5–7	3–4	Throughout Allegheny formation, Pa., Md., W.Va.
9. robustus, n.sp....	20–30	6–10	4–6	20–25	2±	90–120°	6–9	3–5	2–3	2–3	1.5–2	2.5–3	90–100°	5–7.5	3–4.5	Middle Allegheny (Kittanning Group), Md.
10. waldenbergensis (Potonié).....	20–35	8–9	5±	20–30	?	110–130°	6–8	4–5	2	0.5–1.5	2–3	2–3	120–130°	6	3	Waldenberg series (Lower Pottsville), Silesia
11. riparius (Nath.)..	70–80	16	8–9	20	?	180°	13±	4±	2.5±	2.5	1.5	5	125–130°	10–11	5–5.5	Lower Carboniferous (Pocono), Spitzbergen
12. mirabilis (Nath.)..	70+	8–10	3–4	25–35	1.5–2.5	150–165°	7+	2+	2±	0.5	0.5–1.5	3±	140–150°	?	?	Lower Carboniferous (Pocono), Spitzbergen

* All dimensions in mm.

† Infrequently as much as 60 mm.

Camp Miller, Spitzbergen, from beds which have been correlated with the Calciferous sandstone series of Scotland and the Pocono of the Appalachian Province.

Cantheliophorus has a wide geological range which, like the ranges of the several species, may be extended as the result of careful systematic study of other collections, the stratigraphic position of which is known with precision, and it is to be expected that the limits of the normal ranges of some of these dimensions will be somewhat extended, while all of them are likely to be crossed occasionally in the case of supernormal or subnormal individuals.

The genus appears from the evidence in hand to have become extinct about the close of the Allegheny as the result of certain conditions which were unfavorable to arboreal types, for the tree Lycopods were very greatly restricted at this time. The first red beds of the continental period of the Permo-Pennsylvanian appear in Maryland less than 100 ft. above the highest occurrence of *Cantheliophorus*.

Concerning new specific names

In conclusion we feel that we should urge that greater care be exercised in the selection of new specific and even generic names by the paleobotanists who are devoting themselves to the study of structural material. MASLEN (14) in 1899 proposed the specific name *foliaceous* for an unnamed species of *Lepidostrobus* figured earlier by WILLIAMSON (38), overlooking the fact that LESQUEREUX (13) in 1880 had already used this combination. The specific name *Veltheimianus* proposed by SCOTT for a petrified cone of *Lepidostrobus* from the Calciferous sandstone of Scotland, also figured by WILLIAMSON, was employed in the same connection in 1873 by FEISTMANTEL (11) for the impression of an entirely different cone from the Lower Carboniferous of Rothwaltersdorf, Silesia. The name *Lepidostrobus gracilis*, proposed in 1914 by Mrs. AGNES ARBER for a petrified cone from the Lower Coal Measures of Great Britain, was employed in 1853 by NEWBERRY (17) for a cone from Cuyahoga Falls, Ohio, and again in 1877 by SCHMALHAUSEN (25) for a different cone from the Ursa Stufa of Ogur, Siberia. SCOTT and JEFFREY (30) in 1914 published a description of a petrified

cone from the Chattanooga shales of the Waverly Group (Lowermost Mississippian) west of Junction City, Boyle County, Kentucky, which they named *Lepidostrobus Fischeri,* but for this SCOTT has since proposed instead the name *L. kentuckiensis* (29) after ZEILLER had called his attention to the fact that RENAULT (22) had used this combination in 1890 for an impression from the Commentry Basin of France.

Another case to which attention should be called has to do with the use of the term *Lepidocarpon* by SCOTT (26) for the well known seed-bearing strobilus from the Coal Measures of Great Britain, for this name in the form of *Lepidocarpus*, which by the generally accepted rules of nomenclature must be regarded as the same term, has already been employed first by ADANSON in 1763 for a genus of African Proteaceae now included in the genus *Scolymocephalus;* by WEINMANN in 1747 and again by KORTHALS in 1855 for a genus of tropical Rosaceae included now in the genus *Ferolia* Barrère (1741). It is with much hesitation that I disturb a term of such standing as *Lepidocarpon*, and except for yet another case of the prior use of this term generically I should not have done it, for such procedure is certain to cause annoyance and sometimes irritation as well. I refer to the use of the term *Lepidocarpus* by ROTHPLETZ (24) in 1880 for the sporangia of *Lepidodendra* from the Culm of Saxony, so closely comparable to the use made of it by SCOTT that we believe that it should not have been used in its present form in the sense proposed by the latter.

GEOLOGICAL LABORATORY
JOHNS HOPKINS UNIVERSITY

LITERATURE CITED

1. ARBER, Mrs. AGNES, An anatomical study of the Paleozoic cone genus *Lepidostrobus*. Trans. Linn. Soc. II. Bot. 8:205–238. *pls. 21–27*. 1914.
2. BENSON, MARGARET, The sporangiophore, a unit of structure in the Pteridophyta. New Phytol. 7:143–149. *figs. 25, 26*. 1908.
3. BERRY, E. W., Article on Paleobotany in New International Encyclopedia. 1915.
4. ———, The classification of vascular plants. Proc. Nat. Acad. Sci. 3:330–333. 1917.

5. Bower, F. O., Studies in the morphology of the spore producing members, Equisetineae and Lycopodineae. Phil. Trans. Roy. Soc. **185**:473–572. *pls. 42–52.* 1894.
6. ———, Note on abnormal plurality of sporangia in *Lycopodium.* Ann. Botany **17**:278–280. 1903.
7. ———, Origin of a land flora. London. 1908.
8. ———, The quest of phyletic lines. Plant World **15**:97–109. 1912.
9. Brongniart, Adolphe, Observations sur le structure intérieure du *Sigillaria elegans.* Archiv. Museum **1**:405–461. *pls. 25–35.* 1839.
10. Browne, Lady Isabel, The phylogeny and interrelationships of the Pteridophyta. III. Lycopodiales. New Phytol. **7**:150–166. 1908; **8**:51–72. 1909.
11. Feistmantel, O., Zeitschr. Deutsch. Geol. Gesells. **25**:534. *pl. 17. fig. 36.* 1873.
12. Hickling, Geo., Anatomy of *Palcostachya vera* Seward. Ann. Botany **21**:369–386. *pls. 32–33. figs. 1–3.* 1907.
13. Lesquereux, Leo., Coal flora, Pennsylvania. Second Geological Survey, Rept. P. **2**:1880; **3**:1884.
14. Maslen, A. J., Structure of *Lepidostrobus.* Trans. Linn. Soc. II. Ser. Bot. **5**:357. 1899.
15. Nathorst, A. G., Contributions to the Carboniferous Flora of Northeastern Greenland. Denmarks-Eksped. Groenland 1906–1908 **3**:339–346. *pls. 15–16.* 1911.
16. ———, Zur fossilen Flora der Polarländer. Nachträge zur Palaeozoischen Flora Spitzbergens **1**^{6}:1–10. *pls. 1–14. figs. 1–21.* 1914.
17. Newberry, J. S., Fossil plants from the Ohio Coal Basin. Ann. Sci. Cleveland **1**:97. 1853.
18. Potonié, H., Lehrbuch der Pflanzenpalaeontologie. Berlin. 1899.
19. Renault, B., Recherches sur la structure et les affinitées botaniques d'végétaux silicifiés recueilles aux environs d'Autun et de St. Étienne. Publ. Soc. Eduenne. 1878 (pp. 41–50; *pl. 3, figs. 1–7; pl. 4, figs. 8–13*).
20. ———, Structure comparée de quelque tiges de la Flore Carbonifère. Nou. Arch. Mus. d'Hist. Nat. **2**:1879 (p. 270; *pl. 12, figs. 15–19; pl. 13, figs. 1–4*).
21. ———, Course de Botanique Fossile. Paris. **2**:1882.
22. ———, Commentry; Flore Fossile. Paris. 1890 (p. 526).
23. ———, Flore fossile. II, Bassin Houiller et Permiane d'Autun et d'Epinac. Études des Gites Mineraux de la France. 1893 (p. 113; *pl. 56, figs. 5–7; pl. 57, figs. 1, 2*).
24. Rothpletz, A., Die Flora und Fauna der Culmformation bei Hainichen in Sachsen. Bot. Centralbl. **1** and **2**:1880.
25. Schmalhausen, J., Mel. Phys. Chim. Tires. Bull. Acad. Imp. Sci. St. Petersbourg **9**:1877 (p. 632).

26. SCOTT, D. H., Occurrence of a seedlike fructification in certain Paleozoic Lycopods. Proc. Roy. Soc. Lond. **67**:1901 (p. 309).
27. ———, The present position of Paleozoic Botany. Prog. Rei Bot. 1907:137–217.
28. ———, Studies in Fossil Botany. 1:1908 (2d edit.); 2:1909.
29. ———, Note. Proc. Roy. Soc. Lond. B. **88**:1915 (p. 435).
30. SCOTT, D. H., and JEFFREY, E. C., On fossils showing structure from the base of the Waverly Shale of Kentucky. Phil. Trans. Roy. Soc. Lond. B. **205**:1914 (p. 354).
31. TANSLEY, A. G., Reduction in descent. New Phytol. 1:1902 (p. 131).
32. THOMAS, A. P. W., The affinity of *Tmesipteris* with the Sphenophyllales. Proc. Roy. Soc. Lond. **69**:343–350. 1902.
33. WATSON, D. M. S., On *Mesostrobus*, a new genus of Lycopodiaceous cones from the Lower Coal Measures. Ann. Botany **23**:379–397. *pl. 27. figs. 1–6*. 1909.
34. WHITE, DAVID, Stratigraphic succession of the fossil floras of the Pottsville Formation. 20th Ann. Rept. U.S. Geol. Survey. 1900 (p. 825).
35. ———, Stratigraphy and Paleontology of the Upper Carboniferous Rocks of the Kansas Section. Bull. no. 211, U.S. Geol. Survey. 1903 (p. 105).
36. WHITE, I. C., Rept. Prog. Second Geol. Surv. Pennsylvania, Q. 1878 (pp. 51, 54).
37. WILLIAMSON, W. C., Organization of the fossil plants of the Coal Measures. III. Phil. Trans. Roy. Soc. Lond. B. **162**:1872.
38. ———, Same. IX. Phil. Trans. Roy. Soc. Lond. B. **184**:1893 (p. 27. *pl. 9. fig. 27*).

EXPLANATION OF PLATES IX–XI

PLATE IX

FIG. 1.—*Cantheliophorus linearifolius* (Lesq.); Allegheny Formation, Pittston, Pa. (type in Lacoe Coll. U.S.N.M. no. 16069).

FIG. 2.—Same; ×2.

FIG. 3—*C. grandis*, n.sp.; Pottsville Formation, Holmes, W.Va. (collection Md. Geol. Survey).

FIG. 4.—*C. riparius* (Nath.); Lower Carboniferous, Spitzbergen (Nathorst: Nachtr. Pal. Flora Spitzbergens 1914. *pl. 13. fig. 18*).

FIG. 5.—*C. cultriformis* (Lesq.); Allegheny Formation, Cannelton, Pa. (type, Lacoe Coll. U.S.N.M. no. 16087) (Lesq. Coal Flora. III. 1884. *pl. 108. fig. 2*).

FIG. 6.—Same; ×2.

FIG. 7.—Another specimen from same locality (U.S.N.M. no. 16088).

FIGS. 8–10.—*C. linearifolius* (Lesq.); Allegheny Formation, Swallow Falls, Md.; fig. 9 with a part of sporangium in place (Coll. Md. Geol. Survey).

FIGS. 11, 12.—*C. subulatus*, n.sp.; Allegheny Formation, Lansing, Kansas (Lacoe Coll. U.S.N.M. nos. 16095, 16096).

PLATE X

FIG. 13.—Section of stem of *Sigillaria menardi* Brongt. much enlarged (Brongniart: Archives d'Mus. d'Hist. Nat. 1:1839. *pl. 25. fig. 4*).

FIG. 14.—*Calamites* (*Asteromyelon*) *Augustodunense* Renault (Renault: Autun et Epinac. 1893. *pl. 56. fig. 6*).

FIGS. 15, 16.—*Cantheliophorus ensifer*, n.sp.; Pottsville Formation, Swallow Falls, Md. (Coll. Md. Geol. Survey).

FIGS. 17, 18.—*Cantheliophorus* sp.; Lower Carboniferous, Greenland (Nathorst: Groenlands Nord-oestkyst 1911. *pl. 16. figs. 34, 35*).

FIGS. 19–21.—*C. Waldenbergensis* (Potonié); Waldenberg Series, Silesia (Nath. Nachtr. Pal. Fl. Spitzbergen 1914. *fig. 16*, p. 64).

FIGS. 22–24.—*Cl mirabilis* (Nath.); Lower Carboniferous Spitzbergen (Nath. 1914, loc. cit. *pl. 13, figs. 30, 28, 26*); ×2.

FIGS. 25, 26.—*C. robustus*, n.sp.; Allegheny Formation, Westernport, Md.; part of sporangium in place on fig. 25. (×2) (Coll. Md. Geol. Survey).

FIG. 27.—*C. pugiatus*, n.sp.; Pottsville Formation, Campbells Ledge, Pittston, Pa. (Lacoe Coll. U.S.N.M. no. 16108).

FIG. 28.—*C. sicatus*, n.sp.; Allegheny Formation, above Schell, W.Va. (Coll. Md. Geol. Survey).

FIGS. 29, 30.—*C. novaculatus*, n.sp.; Allegheny Formation, Franklin, W.Va.; fig. 29 with attached sporangium partly cut away to expose the sporangiophore beneath; both figs. ×2 (Coll. Md. Geol. Survey).

FIG. 31.—Same species. Allegheny Formation, Luke, Md. (Coll. Md. Geol. Survey).

FIG. 32.—*Lepidodendron* sp. (to be described later); Allegheny Formation, Barrelville, Md.; suspected of being the impression of the stem of the tree which bore *C. novaculatus*.

FIG. 33.—*Calamostachys Decaisnei* Renault (Rech. sur Vegét. Silicifiés. Mem. Soc. Eduenne 1878. *pl. 4. fig. 12*).

PLATE XI

FIG. 34.—Diagrammatic sketch of *Cantheliophorus: a*, toe; *b*, heel; *c*, keel; *d*, sporophyll pedicel; *e*, blade; *f*, inner or axillary field; *g*, outer field; *h*, brace; *i*, crest; *k*, guard; *l*, beak; *m*, angle of inflexion, blade to pedicel; *n*, angle of inclination, guard to pedicel; *o*, sporangium; *p*, point of inflexion; *q*, wing of pedicel.

FIG. 35.—Same, seen from above.

FIG. 36.—Diagrammatic cross-section (tangential) through sporangia borne by single sporangiophore of *Calamostachys Decaisnei* Renault, showing relation of sterile tissue (black) to sporogenous tissue.

FIG. 37.—Similar section for *Cantheliophorus*.

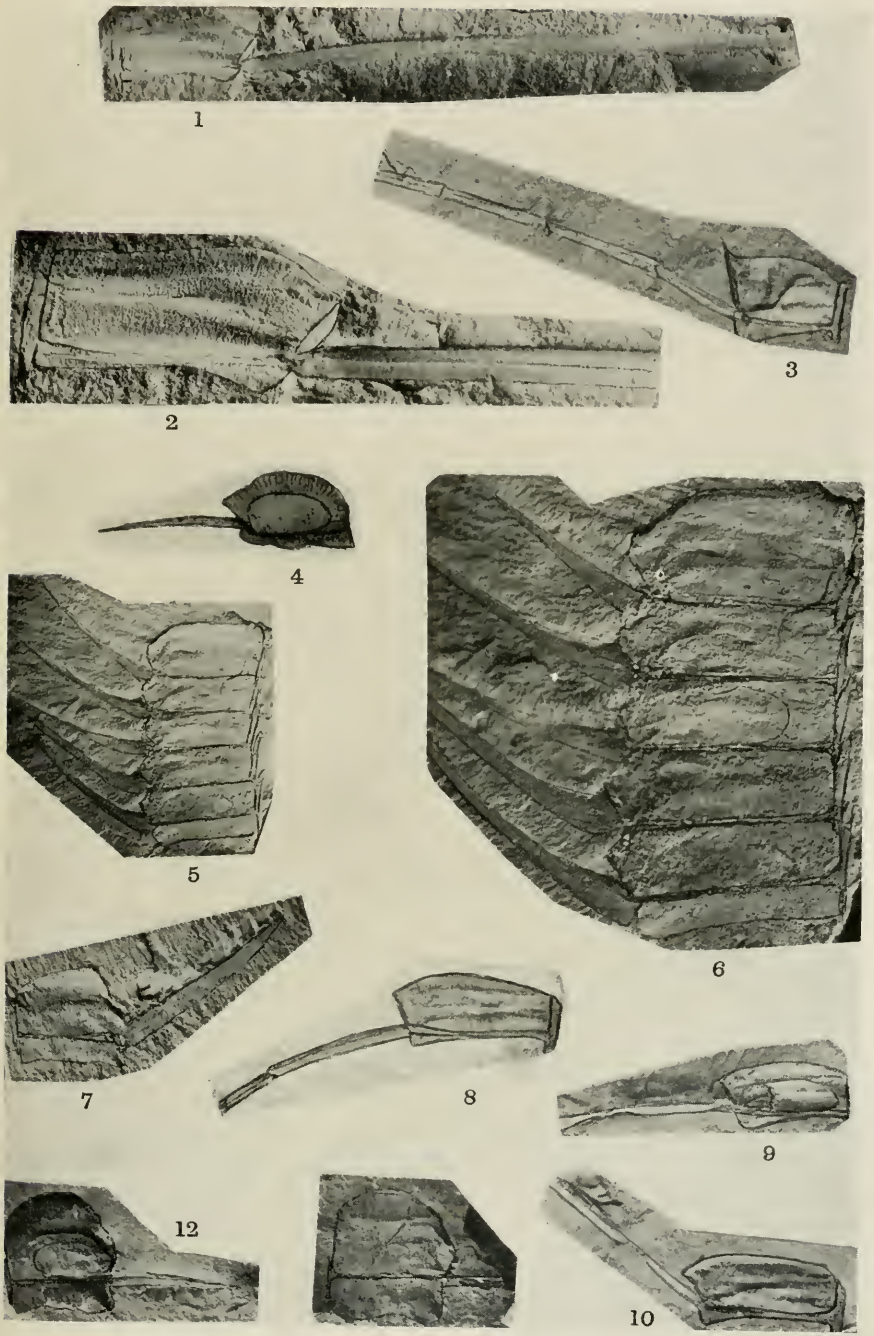
FIG. 38.—Same for *Lepidostrobus*.

FIG. 39.—*Lepidostrobus Mazocarpon*, after Miss BENSON; microsporangium in cross-section, ×15 (New Phytol. **7**: 1908. *fig. 26*, p. 145).

FIG. 40.—*Mazocarpon*, after Miss BENSON, ×15. (loc. cit. 1908. *fig. 25*, p. 144).

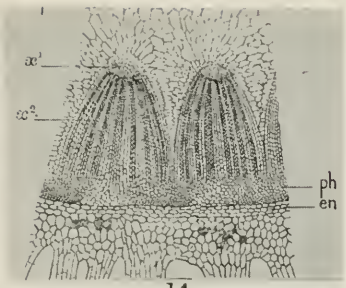
FIG. 41.—*Lepidostrobus Veltheimianus* Scott, after WILLIAMSON (Phil. Trans. Roy. Soc. Lond. 1872. *pl. 44, fig. 25*); ×22.

FIG. 42.—*Lepidostrobus Oldhamius* Williamson, after Mrs. AGNES ARBER (Trans. Linn. Soc. Bot. II. **8**: *pl. 21. fig. 1.* 1914); ×7; section obliquely through apex of cone illustrating the sterile tissue within the immature sporangia.



BASSLER on CANTHELIOPHORUS

13

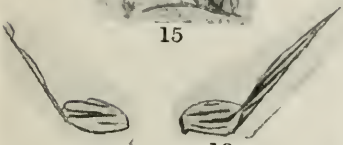


14

15

22

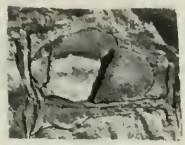
24



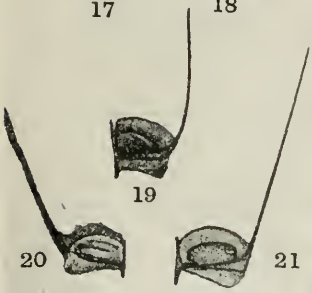
17 18

23

32



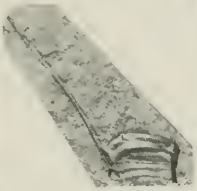
25



19 20 21

16

26



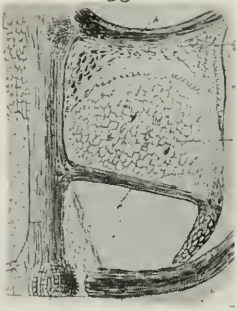
27

28

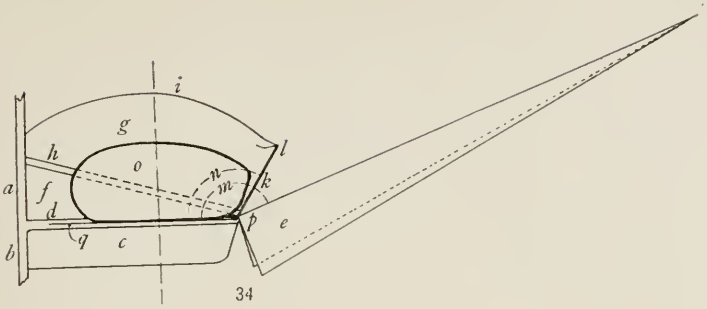
29

30

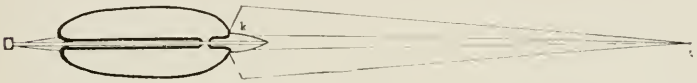
31



33

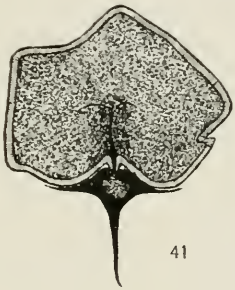


34

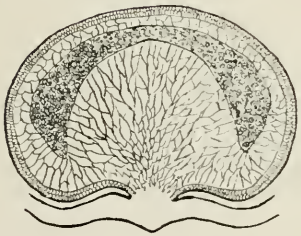


35

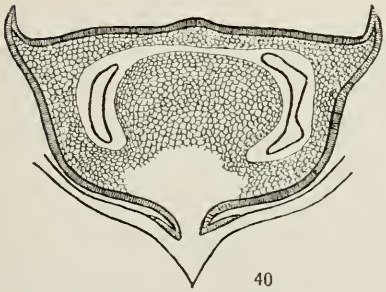
36 37 38



41



39



40

42

BASSLER on CANTHELIOPHORUS

INTERSEXES IN PLANTAGO LANCEOLATA

A. B. Stout

(WITH PLATES XII, XIII)

Through the resent researches of Goldschmidt (11, 12, 13), Banta (1), Whitman, Riddle, and their associates (see especially summaries by Riddle 22, 23), and Lillie (17, 18), the facts of intersexualism have acquired a significance which must be considered by any theory of sexuality and sex determination. These new studies show that in widely separated groups of animals which are usually dioecious various grades and degrees of maleness and femaleness in a single individual are common. Judged as entire individuals, such "intersexes" or "sex intergrades" may be predominately male or female, or there may be various grades in the relative development of maleness and femaleness, giving in some cases at least functional hermaphrodites. Along with these there may be individuals that are only male or female. An individual sex organ may start development as of one sex and change to the other, or there may be a decidedly simultaneous development of male and female sex organs, as in the fully functional hermaphrodite. The more remote secondary sex characters also exhibit characteristics of maleness, femaleness, or various grades of modifications that are intermediate.

Such development of intersexuality in forms usually considered as dioecious is evidence that even in dioecious forms sex is not necessarily determined at fertilization, and that sex is not alternative and irreversible for an individual or even for a sex organ. The data are particularly suggestive of the probability that sex differentiation in dioecious forms and in hermaphrodite forms is essentially the same process, and thus that sex determination is on the same fundamental basis in both plants (which are prevailingly hermaphrodite) and animals (which are prevailingly dioecious).

In plants the most intimate association of the two sporophytic sex organs is seen in the so-called perfect flowers. The opposite

extreme is seen in dioecious species. Various grades of sexuality intermediate between these two are seen in species classed as monoecious and polygamous, most striking of which are the numerous instances where all grades of sexuality are to be seen among the various flowers produced by a single individual. DARWIN (7) presents an excellent summary of these cases as evidence that "various hermaphrodite plants have become or are becoming dioecious by many and exceedingly small steps" (p. 181). DARWIN was not directly concerned with the problem of sex determination. He was seeking to discover methods and principles of evolution. In his discussion of sex heteromorphism he places much emphasis on the law of compensation in the utilization of the energy at the disposal of plants, and thus gives recognition to a metabolic theory of sex determination in so far as it relates to the development of the floral organs.

There has been no special dispute over the very obvious fact that the condition of hermaphroditism indicates that sex differentiation may arise through somatic differentiation. According to the sex chromosome theory, however, sex in dioecious species is assumed to be determined qualitatively in reduction divisions and in fertilization, and that the two sexes are hence alternative and represent fundamentally irreversible conditions. In developing this theory, however, little attention has been paid to hermaphrodites, and in view of their predominance in plants the theory cannot be regarded as expressing any broad biological law.

The recent investigations of GOLDSCHMIDT, BANTA, RIDDLE, and LILLIE show that the sex of dioecious species is not necessarily irreversible. This is especially striking, as to demonstration in pedigreed cultures, in the results obtained by BANTA. By means of parthenogenetic reproduction he propagated races from females of *Simocephalus vetulus* for 130 generations, getting nothing but female individuals, only to have the femaleness break up in the 131st generation, giving males, females, hermaphrodites, and many grades of intersexes.

Turning to plants, we have such striking cases of changes of sex combined with conditions of intersexuality as are recently reported by DAVEY and GIBSON (8). They have studied the sex

of the bog myrtle or sweet gale (*Myrica Gale*). This plant is a small shrub which grows abundantly in swamps and heaths in Europe, Asia, and the northern part of North America. The species is described as strictly dioecious, and until 1901 no observations that it is ever otherwise have apparently been recorded. DAVEY and GIBSON find that in the peat moors of England there are everywhere present intersexes, or, as they call them, "mixed plants" of many gradations. Judged as a whole, the plants present every gradation of intersexes between dioecism, monoecism, and hermaphroditism. The variations seen in the various catkins on a single plant include the entire range, and all the grades may appear among the flowers of a single catkin. Furthermore, a study of individual plants for a series of years shows that changes of sex from year to year occur. Plants entirely female in 1913 were entirely male in 1914. Plants female in 1913 were mixed in 1914, entirely or nearly all male in 1915, and again female in 1916.

DAVEY and GIBSON point out that the changes in sex seen in *Myrica Gale* indicate that sex determination is here in some way associated with environmental conditions. In regard to this they state as follows:

> The conditions which naturally suggest themselves are moisture, temperature, and light (with their influence on nutrition), and also the previous state of a plant as regards the production of fruit. Since the staminate flowers are developed early in the season preceding that in which they flower, while the pistillate catkins develop much later, it is possible that conditions accelerating or retarding the development of catkin buds may influence the proportions of the two kinds (pp. 150–151).

The facts reported for *Myrica Gale* are striking and suggest that similar conditions may already be present or may spontaneously arise in other species now considered as dioecious.

Intersexes in Plantago lanceolata

This species is a native of Europe and Asia. It has been introduced into America, where it has spread from the Atlantic to the Pacific, through Canada, and southward in the United States to Florida. It is well known as a vigorously growing species which in many sections has become a troublesome weed.

Standard botanical treatments describe this species as having only perfect flowers. For over 50 years, however, sex polymorphism has been recognized as present in the species. The tendency has been to group the individuals in 3 classes (LUDWIG 19), most recently designated by BARTLETT (2) as (1) first form hermaphrodite, (2) second form hermaphrodite, and (3) female. CORRENS (4), however, groups plants of this species grown from seed collected near Leipzig, Germany, into 5 classes, in two of which there was variation in single spikes (*a*) from hermaphrodite flowers to flowers with imperfectly developed stamens (≒☿), and (*b*) from more or less perfect flowers to flowers only female (≒☿ and ♀). In thus making these classes recognition is given by CORRENS to variations in sex organs which include various grades of gynomonoecism already observed in this species by SCHULZ (24).

The difficulty of making any adequate classification, expressed in some degree by CORRENS (4) and by BARTLETT (3), has been very apparent from the observations which the writer has made. In 1912 BARTLETT very kindly supplied me with plants which he classed under the 3 forms just noted. Seed progenies have been grown and observations made of plants growing wild in the fields in and about the New York Botanical Garden, where *P. lanceolata* is exceedingly abundant. Study of this material reveals that there is present a wide range of variations in the development of sporophytic sex organs, which in its general aspects is quite identical with the phenomenon of intersexualism especially described by GOLDSCHMIDT, BANTA, and by DAVEY and GIBSON.

DESCRIPTION OF THE THREE FORMS

Flowers typical of the forms most generally recognized may first be described, as illustrating the two extremes and one intermediate. The flower drawn for a plant was in all cases selected from the middle portion of a spike, and was typical of a large number of flowers in bloom. The flower was placed on a glass slide, a large cover glass was placed over it to bring the various parts into somewhat the same plane, measurement was made of the flower parts under very low magnification by ocular microm-

eter, and all parts were then drawn to scale. Stamens and spores were measured and drawn under higher magnifications.

First form hermaphrodite (figs. 1–3, 49, 50).—This term has been applied by Bartlett (2) to plants whose flowers very uniformly show most complete development of stamens. The filaments are usually twice as long as the pistils. The anthers are large and well developed and white in color. In face view when freshly dehisced (fig. 2) they measure about 2 mm. in length by 1.5 mm. in width. The corolla lobes are well developed, with blades strongly reflexed when anthesis is complete.

The pollen of numerous plants of this form was examined microscopically and rather extensive germination tests were made.[1] Perfect grains are almost spherical, with thin smooth walls and granular contents. There is much variation in the size of grains that appear to be perfect, the smallest being about one-third the diameter of the largest. There is also a considerable number of obviously imperfect grains with shrunken shriveled walls that are either empty or have hyaline contents. Such grains do not swell up when placed in water or in various media used in testing germination. Impotent grains frequently constitute 25 per cent of the pollen of a microscopical mount. They have always been found present to some extent.

After a rather extended series of experiments it was found that the pollen of this form germinates well in sugar-agar media. The most uniformly favorable results were obtained with a medium of 15 per cent sugar to which 3 per cent agar was added. Good germination was also obtained in 15–1 and 15–5 solutions. The largest tubes observed measured 3.1 mm. in length. Even in the case of the most complete germination not all the spores with granular contents germinated. No shriveled and hyaline spores germinated, but some of the smallest of the apparently perfect spores germinated. Some granular spores of all the sizes failed to germinate. A series of countings was made for a plant

[1] In the various studies of the germinations of pollen reported in this paper the writer has been assisted by Lieut. M. V. Reed, a former student and scholar at the New York Botanical Garden, and by Miss Helene M. Boas, laboratory assistant, for whose efficient aid and cooperation acknowledgment is here made.

whose pollen germinated most completely. Of 1003 pollen grains placed in 15–1 and 15–3 sugar-agar media, 147 grains (about 15 per cent) had failed to germinate at the end of 24 hours, and of these about half were shrunken and hyaline.

Data regarding the ability to produce seed are of interest in bearing on the condition of femaleness in intersexes. This is a point of particular interest in respect to plants classed as hermaphrodites. LUDWIG (19) reports that the reduction in stamens seen in female plants of *Plantago* is associated with increased fruitfulness. I have made special observations on 3 plants of the first form. In 1916 all of these failed to set any seed to controlled self-pollination. In 1917 two of these failed to produce seed to free open pollination; the third plant was isolated with a pistillate plant. Day after day pollen of the hermaphrodite was very generously shaken over stigmas of both plants. The female plant produced an abundance of seed, while not a seed developed on the other. It is possible that physiological self- and cross-incompatibilities may be operating here (STOUT 25), but the various grades of impotence and intersexuality seen in stamens of plants of this species suggest that the failure to set seed when pollinated with viable pollen may involve impotence of pistils. It is readily observed in the field that many plants fail completely to set seed; although pistils are present they may be incapable of functioning. Such plants classified as of first form are functionally male only. In the highly developed stamens and impotent pistils these plants may be considered as representing the extreme of maleness seen in this species. Some first form plants, however, produce seed in abundance.

SECOND FORM HERMAPHRODITE (figs. 4–6, 53).—Plants most typical of this class, as thus designated by BARTLETT (2), are especially to be distinguished from the first form by the stamens, which have shorter filaments and slender yellowish-green anthers. In most cases the anthers do not dehisce. There is no excessive development of sterile tissue in the stamens. Pollen grains are numerous, but the largest are only about half the diameter of the largest of the first form; but poor and shrunken grains appear to be no more numerous. Attempts to germinate the pollen have

been unsuccessful. Pollen grains have been removed from anthers of various ages, anthers have been artificially dried to various stages of dryness before pollen was removed, and many kinds of media have been employed. In extensive tests of pollen from 4 different plants during 2 years of bloom only one germinating grain was found, and this may have been accidentally introduced from another plant.

Accurate tests of the ability to set seed have not been made for plants that are best classed with this form. From the evidence at hand it appears that the pistils are very frequently functional, so that the plants most typical of this class are functionally female.

It will be noted later that the flowers of numerous plants which would ordinarily be classed with this form are found upon more careful examination to present somewhat decided differences indicative of various grades of maleness.

Female or pistillate form (figs. 7–10, 58).—Plants that may be grouped in this class have flowers with rudimentary and rather reduced stamens, the tips of which only slightly or not at all protrude above the corolla. There is much variation, however, in the development of the stamens in such flowers. Frequently there is a differentiation of filaments and anthers as shown in fig. 8, and in cases even some traces of the 4 anther sacs. In other cases the stamens are more foliose, with no trace of anthers, as shown in fig. 10.

Numerous plants with this general type of stamen have what may be termed "closed" flowers; that is, the corolla lobes do not spread out and become reflexed, and when the flowers are fully developed they appear as shown in figs. 7 and 9, a condition decidedly in contrast to the reflexed corollas seen in such flowers as shown in figs. 1 and 4. Such a reduction of corolla in pistillate plants has long been recognized in gynodioecious species, and such a condition was recognized for *P. lanceolata* in the early observations made by Darwin (**7**, p. 307) and Ludwig (**19**, p. 322). Examination of such flowers shows that the blade portion of the petals is well developed, but that the part below the blade is shortened and often crumpled; the corolla lobes, therefore, are

not pushed up above the calyx lobes. The writer has examined at least 100 plants with this closed corolla type of flowers. In every case the stamens were scarcely or not at all exserted and were completely composed of sterile tissue.

Thus far all plants that I have seen which had completely sterile non-exserted stamens also had closed flowers; but the pistillate form as described by BARTLETT also includes plants with corollas fully developed and reflexed, and such a flower is figured by him (2, fig. 3) as illustrating a typical pistillate flower. Various plants with expanded petals and completely sterile or indehiscent stamens are potentially only females. The rudimentary development of stamens and the character of the corolla may be regarded as extreme cases of loss of maleness, and the character of the corolla may be considered as a secondary sex character associated with femaleness and appearing when maleness is most completely lacking.

From general observations of plants in the field and in a greenhouse, and from such controlled pollination as have been made, it appears that plants of this pistillate type are highly productive of seed. A few plants, however, have set no seed when exposed to favorable conditions for free cross-pollination, which suggests that the pistils of some of the pistillate plants may be impotent.

These descriptions refer to the types of flowers that characterize the 3 forms most generally recognized, and into which attempts have been made to classify all individuals. Both CORRENS (4) and BARTLETT (3), however, recognized that it was somewhat difficult to thus place all individuals observed by them. Such a difficulty has been very apparent in respect to the material studied by the writer. The variations present almost every grade of intermediates between the two extremes described, and seem to involve a series of sex intergrades or intersexes. The character of flowers may be quite uniform for a plant as a whole, or there may be a wide range of intersexuality among the different flowers of a single spike, or even among the various stamens of a single flower. Flowers typical for some of these may be described and arbitrarily numbered as follows:

INTERSEXES WITH FLOWERS UNIFORM

No. 11 (figs. 11, 12, 50).—The relative lengths of pistils and stamens in the flowers of this plant are quite as in the first form. The general appearance of the spikes in full bloom is quite similar (fig. 50), but the anthers are noticeably smaller and more narrow, and they are slightly greenish-yellow in color. Many anthers do not dehisce, and after 2 or 3 days they turn brown. A high percentage of pollen is impotent, but the size of the apparently good grains ranges quite as for the first form.

No. 13 (figs. 13, 14).—The stamens produced by this plant are somewhat smaller than those of the first form. They are slightly greenish-yellow, but are fully dehiscent. A large proportion of pollen was impotent, but a few well formed grains as large as the largest of the first form were found. Tests of pollen germination in 15–1, 15–3, and 15–5 sugar-agar media gave germination in about 3 per cent of the grains. The tubes made a feeble growth and the longest obtained measured only 0.08 mm.

No. 15 (figs. 15, 17).—Pistils of this plant are normally longer than the stamens when both are fully developed. The filaments are only slightly shorter than in the first form; the anthers are decidedly smaller, but all are white and fully dehiscent. A large proportion of the pollen is impotent, but normal grains of large size are abundant. The pistils produced by this plant were among the longest observed on any plant, except for the abnormally elongated pistils (fig. 56) which appear in plants under certain conditions.

No. 18 (figs. 18, 19).—The stamens and pistils in flowers of this plant are of nearly equal length. Nearly half of the apical portion of the stamens is composed of a sterile blade. The small anther sacs, however, are well developed and fully dehiscent. Scarcely a shriveled pollen grain was found, the grains being very uniformly of large size and a high percentage of them being viable. In this plant the amount of sterile tissue in stamens is decidedly more than that seen in nos. 1, 11–17, but there is better development of such sporogenous tissue as is formed.

No. 20 (figs. 20–22, 54).—At the time when the pistils were receptive the flowers of this plant appeared as shown in fig 20,

with the lobes of the corolla scarcely expanding and the large anthers scarcely protruding. Several days later, when the stigmas were beginning to shrivel, the corolla was slightly expanded (fig. 21). The anthers are large, but there is marked inequality in size of the 2 pairs. The pair next to the insertion of the filament is uniformly the larger and overlaps somewhat the smaller pair, so that in face view an anther appears as in fig. 22. Dehiscence is somewhat irregular and is confined to the apex, so that few spores are shed. The anthers persist until all the flowers in a spike bloom. In old anthers the microspores are dry and shriveled, but in fresh anthers they are mostly of large size and appear to be normal; but no germination was obtained in cultures.

No. 23 (figs. 23, 24).—A plant with short crinkled filaments and extremely narrow and pale green anthers. Most anthers dehisce fully. Very few microspores are plump and have granular contents. The range of size of grains is quite as for the first form, but no germination was obtained in cultures.

No. 25 (figs. 25–27).—This plant resembles a second form hermaphrodite. The stamens, however, are decidedly shorter, the anthers are somewhat of the same shape but dehisce regularly, and the microspores range to a larger size quite as for the first form. About 20 per cent of the pollen grains tested germinated, but in all cases the tubes made only a very feeble growth.

No. 28 (figs. 28, 29).—The stamens produced by this plant have short and crinkled filaments with decidedly green anthers. The apical half of the anthers is composed of a sterile green blade, and the anther sacs are much reduced in size and are not dehiscent. At least 75 per cent of the pollen grains that are produced are of large size and are plump with granular contents. In 3 cultures of pollen removed from fully developed anthers 6 grains germinated and the best developed tube was 0.60 mm. in length.

No. 30 (figs. 30, 31).—In general appearance the stamens produced by this plant resemble those of the second form; the anthers are greenish yellow but the filaments are shorter. There is a marked peculiarity, however, in the development of anther sacs not observed thus far on any other plant. When anthers

are fully extended their appearance suggests dehiscence, but an examination at earlier stages of development shows that the 4 anther sacs develop as thin platelike and chiefly indehiscent structures, with only a few scattering thin areas of sporogenous tissue.

No. 32 (figs. 32, 33).—The stamens of this plant protrude only slightly above the throat of the corolla. The general shape of the anthers is maintained, but the anthers are wholly or nearly wholly sterile, and there are only slight irregularities on the surface suggestive of any differentiation of anther sacs.

Nos. 34 and 35.—Numerous plants are to be found having stamens with no trace of sporogenous tissue or even of anther differentiation. When such rudimentary stamens are short, they may be entirely or nearly inclosed within the corolla as previously described for certain plants classed as pistillate (figs. 9, 10). In many cases, however, the stamens are more extended and take on the character of leaves, both as to general shape and color. One of the cases best developed in this direction is illustrated in fig. 34. In fig. 35 the foliose stamens were of nearly uniform width and were much recurved.

Summary.—It is difficult to arrange or classify the flowers typical of individual plants, such as described, in any fully consistent series. Various types of flower and various grades of development of stamens are to be recognized, and it is evident that as arranged in descriptions and in plates the flowers of nos. 11–32 comprise a series which presents a quite continuous gradation between such extremes as shown in figs. 1 and 7. Stamens decrease noticeably in length of filaments, in size, in shape, and in dehiscence of anthers, in the relative amount of tissue that is sporogenous, and in the total number and viability of microspores produced. Complete absence of sporogenous tissue is seen in no. 32, almost complete absence of such tissue is seen in no. 30, and indehiscence is complete in no. 28, giving plants that can function only as females. Reduction in size of anthers and of the amount of sporogenous tissue, however, does not necessarily involve also a decrease in size and viability of the spores which are produced, as is shown in no. 18. Marked differences in viability of pollen are in evidence. Rarely was any germination

observed in microspores artificially removed from indehiscent anthers. Very feeble germination was also obtained in tests of large sized grains from fully dehiscent anthers, as in no. 25. In many plants completely sterile stamens retain some suggestion of filaments and anthers in regard to the general form, but all traces of such differentiation may disappear, giving only foliose structures as shown in nos. 10, 34, and 35. In general, the various grades of development of stamens may well be regarded as indicating different grades of maleness.

In the case of all plants the flowers of which have here been described and illustrated, observations were made of flowers in numerous heads throughout at least one season of bloom. Some have been under observation for several years. At the extreme tip of the spikes in many plants there is a tendency for flowers to develop poorly; the pistils usually protrude, but the stamens are poorly formed and often flowers fail to open. This is the tendency to gynomonoecism especially emphasized by SCHULZ (24) and CORRENS (4). This tendency is evidently more marked in some plants than in others, but I am unable to make any classification on this basis. For the plants already discussed the flowers were very uniform for at least four-fifths of the spikes, as indicated in the spikes (excepting no. 55) shown in pl. 13.

GRADES OF INTERSEXUALISM

Variations in the development of stamens in the same flower or among sister flowers are frequent for many plants. In such cases there are various mixtures of different types of flowers and stamens. Some of these may be noted as follows:

No. 36 (fig. 36).—For this plant some stamens were nearly identical with those of the first form, while others were quite as in the second form. Differences in the length of stamens in a single flower were conspicuous. Some anthers failed completely to dehisce, while others dehisced fully. The greater portion of the pollen was impotent. Grains of large size were present and some of these from dehiscing anthers germinated well in cultures.

No. 37 (figs. 37, 38, 52).—As in the plant previously noted, there is much variation in the length of filaments among stamens

of the same flower. Here, however, the anthers are all quite uniform in size and shape. A rather large portion of the apex is sterile, but the anther sacs dehisce fully, and about 50 per cent of the pollen which they contain appears to be normal. In tests, however, only grains of large size germinated, and the tubes from these made only a feeble growth.

No. 39 (figs. 39, 40).—Filaments are here not only of unequal length, but all are more or less twisted, and nearly all are expanded broadly at the base of the anthers. The upper portions of the anthers are leaflike. Anther sacs vary in number and in degree of development; all 4 may be in evidence, or there may be only 2 (fig. 40), but all are more or less rudimentary and none dehisce. Nearly 30 per cent of the microspores examined were granular and of large size. Of 3 cultures, only 2 grains germinated, and the best tube obtained was 0.35 mm. in length.

No. 41 (figs. 41–43).—In the stamens of this plant the anthers are reduced to irregularly sagittate-shaped leafy structures. Such structures are often composed only of sterile tissue; in some a mere nest of spores develops, but these spores are completely imbedded in sterile tissue. In no case was more than one such nest found in a stamen. Dissection of fully mature structures revealed that the microspores were represented by shriveled cells (fig. 43).

No. 44 (figs. 44, 45).—In this plant the stamens are somewhat more leaflike than those just described. Some are completely sterile, and usually one nest, but sometimes two, of sporogenous tissue may be present in a stamen. Only a few pollen grains appear normal when dissected out.

No. 46 (figs. 46, 47, 48, 56).—A wide range of variation is seen among the stamens produced by this plant. All stamens in a flower may be completely sterile and foliose, as in fig. 46, all may have quite well developed anthers with much good pollen, or all grades between these extremes may be present; 4 stamens from a single flower are shown in fig. 48, and illustrate very well this range. Flowers growing side by side and opening at the same date exhibit wide variations and a great mixture of types. In several plants under observation this was the condition in all

spikes throughout the entire period of bloom. The general appearance of typical spikes from such plants is shown in nos. 55 and 56, but the wide range of stamen forms which are present is not clearly shown. The spikes shown in no. 56 also show the excessive growth which stigmas frequently make. It has been suggested that this occurs when pollination and fertilization have not been effected, and that successful pollination inhibits such growth. It is possible, however, that such growth is an indication of loss of femaleness. Studies are now in progress to determine especially the functional potentiality of pistils of plants for which this phenomenon is very general.

SUMMARY.—The flowers described and illustrated for plants 36–47 show that wide variations exist in the development of stamens among various flowers of a plant, or even among stamens of a single flower. The range is in some cases almost identical with the extremes seen for plants as wholes (nos. 1–35). This statement refers to the flowers produced in the lower two-thirds of the spikes. It may be noted that the range is greater for such a plant as no. 46 than for one like 36 or 37. This variation is not identical with the tendency for the last flowers of a spike to be different from earlier flowers. Here there is a marked mixture throughout the spikes.

NATURAL DISTRIBUTION

In the fields in and about the New York Botanical Garden *P. lanceolata* is so abundant that it often dominates the vegetation over a considerable area. Here plants that approach the first form are most numerous; female plants corresponding to the type described as nos. 7 and 9 are abundant; and there are thousands of plants which are in some degree intermediate between these extremes. Many plants with mixed flowers are to be found. With respect to vegetation characters and to general size and shape of spikes, extremely wide variations are everywhere in evidence.

The variations in flower forms noted by DARWIN for England, by LUDWIG and CORRENS for Germany, and by BARTLETT in the vicinity of Washington, D.C., indicate that much the same range

of variation is to be seen over a wide geographical area. Undoubtedly many of the plants classed as intermediate gynomonoecious, especially by CORRENS, present a range of variations quite identical to those here described.

The wide geographical range of this species, and especially its recent rapid spread in America, give opportunity to observe to what extent there is geographic distribution of races possessing distinctive differences in sex heteromorphism.

Discussion

The term intersexuality, as especially applied by GOLDSCHMIDT to conditions of sex in *Lymantria dispar*, can with equal adequacy be applied to such sex variations as are evident in *Plantago lanceolata*.

It must be recognized that the significance of such variations is to be sought in the conception that there may be different degrees in the expression of maleness and femaleness. Cases of intersexuality afford material for the study of stages and degrees of sexuality and sex determination.

The observations reported for *P. lanceolata* refer almost entirely to maleness. The variations in development of the stamens, with their anthers and contents, are easily and directly to be observed. Evidences of marked variations in the development and functioning of the pistils are also in evidence, and further studies of femaleness are in progress.

It is very evident that there is a wide range of variation in the degree in which maleness is expressed. Measured by the amount of sporogenous tissue, there is every degree of sexual development between the highest grade seen and complete sterility. The size of the stamen as a whole and the size and shape of its various parts exhibit a series from the normal to extremely rudimentary structures. There are two forms in which this decrease in maleness is expressed. In one the stamens are greatly reduced in size; in the other they become foliose. The foliose character is seen first in the slight enlargements of the sterile tissue at the apex of the anther, as shown in figs. 19 and 29.

It is to be recognized that the impotence of one or the other of the sex organs involved in intersexuality is to be distinguished

from sterility of the type classed as impotent (STOUT 25), which results very frequently from hybridization. In sterility of hybrids there is poor development of both sets of sex organs; stamens and pistils are both affected very uniformly, and the tendency is to give complete sterility. In intersexuality loss of sex development for one sex is not necessarily associated with similar loss in the expression of the other sex. In fact, the opposite condition is the normal one for such cases.

In *P. lanceolata* the so-called "first form" is very high in its grade of maleness, and it is in these plants apparently that seed production is noticeably low. As already stated, such plants may fail to set any seed. They have maleness well developed, but functional femaleness may be lost, although pistils are present. Likewise in the most marked cases of loss of maleness the degree of femaleness may be high, as is seen in the plants classed as females. DARWIN (7) reports that females in certain gynodioecious species (*Thymus serpyllum*, *T. vulgaris*, and *Satureia hortensis*) are much more productive of seed than the hermaphrodites, and that thus the species produces more seed than if all were hermaphrodites, a condition to which he attaches evolutionary significance in the formation and separation of the two sex forms. CORRENS (5), however, reports that the hermaphrodites of *S. hortensis* are more productive of fruit and seeds than the females.

If it is found that in *P. lanceolata* femaleness also varies in the degree of its expression, it is quite probable that increased maleness is correlated in the individual with decreased femaleness. Still it is also possible that the variations are such that both decreased maleness and femaleness may be present in the same individuals, that individuals may be intermediate for both, and that both maleness and femaleness may be well developed, giving full hermaphrodites. All these conditions, it appears, are represented in the groups of intersexes studied by GOLDSCHMIDT, BANTA, and by DAVEY and GIBSON. Such facts go far toward establishing the fundamental similarity between sex characters and every other class of structures as functional hereditary characters.

It is the tendency to a differential loss of one sex that distinguishes intersexuality from sterility (impotence) resulting from

hybridization, and from that sterility ascribed to replacement of sexual reproduction by asexual means (GATES and GOODSPEED 9), in both of which the tendency is to give a very uniform impotence of both sexes. A high degree of impotence is present in many plants regarded as pure species. JEFFREY (14, 15, 16) has recently emphasized the view that such sterility is to be considered as conclusive evidence of hybrid origin. Intersexuality, however, involves much impotence, and may very clearly develop in pure species through lability of the processes of sex determination.

At this point one may well inquire whether differences in sexuality somewhat akin to intersexuality may be present in species that are morphologically fully hermaphrodite, and in which no appreciable impotence of sex organs is in evidence. For example, DARWIN reports that plants of "the short-styled form of *Primula veris* produce more seed than the long-styled in the proportion of nearly four to three (7, p. 19), and that in *Lythrum Salicaria* (6, 7) the mid-styled form is potentially capable of higher seed production than plants or either of the other two forms. Judged on the basis of seed production, certain forms in heterostyled species appear to be more female than others. Sexuality of species as such is obviously more intense in some than in others if we are to judge by seed reproduction. Much variation in total seed production is seen among races and among individuals of a race. Such considerations raise many questions regarding the determination of potentiality of sex reproduction through production of seed, and most especially of the relations of vegetative to reproductive function. Undoubtedly much variation in maleness and femaleness exists in sex organs that are morphologically perfect. The sexual behavior of female pigeons has especially been studied by RIDDLE as an index of the degree to which femaleness is developed. He states (22, p. 341) that "females hatched from eggs laid earlier in the season are more masculine in their sex behavior than are their own full sisters hatched later in the season. *And several grades of females can be thus seriated according to season of hatching.*"

The existence of physiological incompatibilities (STOUT 25) between sex organs that are fully formed, potentially functional,

and of simultaneous development are especially well revealed in self-fertilization of numerous species that are homomorphic hermaphrodites. Judged by ability of sex organs to function together, both femaleness and maleness of sex organs are seen in such cases to be of various grades of intensity. Such cases reveal that grades of functional or physiological sexuality may be quite independent of morphological sexuality. The striking feature of incompatibilities, however, is that sex organs which are functionless in some relations are highly functional in certain other relations. For example, it is not complete loss of femaleness, but only a loss in relation to certain degrees or grades of maleness.

The conditions that exist in *Campanula carpatica* (PELLEW 21) are of special interest in indicating that variations in the relative development of sex organs and physiological incompatibilities may both operate in a single species. PELLEW finds that there is a wide range of variations from normal hermaphrodites to females quite as I have described in *P. lanceolata;* it is also reported that nearly all hermaphrodites are self-sterile (physiological incompatibility). The "self-sterile" hermaphrodites used in the experiments set seed to cross-pollination, but the extent to which self- and cross-incompatibilities may be operating among hermaphrodites and in crosses of hermaphrodites with females was not determined, and the studies do not reveal whether or not some plants classed as hermaphrodites may be impotent as to femaleness.

The inheritance of various grades of intersexes in *P. lanceolata* is a problem under investigation, and a discussion of the researches (CORRENS, BARTLETT, GOLDSCHMIDT, RIDDLE, PELLEW, etc.) bearing on this question therefore will not be made here.

It is quite clear that sex differentiation is to be considered as morphological and as physiological. Physiologically the essential and only index of sex in cells is the capacity for their fusion which culminates in the expression of that function by sex cells. It is in decided contrast to that property of asexuality which is seen in cell division and cell growth.

Morphological sexuality consists purely and solely of adaptations to facilitate the bringing into juxtaposition cells that are

capable of fusion when juxtaposed. It may consist of (1) the more or less immediate modification of physical structure of the cells (in spermatogenesis and oogenesis) that are to fuse, and (2) of modifications of organs associated with the development of sex cells, either in the sporophyte or the gametophyte, or both. All of these latter are in reality secondary sex characters; true primary sex characters are to be considered as belonging to the cells that fuse, a view clearly stated by STRASBURGER (26).

The relationship between morphological and physiological sex differentiation is well shown in the flowering plants. We may take a hermaphrodite with perfect flowers as a type. Primarily such a plant is a spore-producing individual; it is a sporophyte in which heterospory is in evidence. The stamens bear microspores, the pistils bear macrospores. These spores are asexual in that they are not able to fuse. They are sexual, however, to the extent that sex is here already determined. Anatomical expression of maleness and femaleness here appears in sporophytic structures, and the particular sex of the future generations of cells in asexual descent is predetermined until the next fusion of sex cells or the development of a sporophyte through apogamy. The pollen grains grow into microgametophytes producing male sex cells or sperms. The macrospores grow into the macrogametophytes which produce the eggs. The alternation of generations is marked; the one is sporophytic; the other is gametophytic. But maleness can be traced back through the pollen tube, through pollen, beyond reduction divisions, to the beginning of somatic differentiation of stamens. Likewise femaleness can be traced to the beginning of the organogenesis of the pistil. These facts certainly justify the application of the terms male and female to structures that in their morphology are sporophytic. This view has frequently been criticized by those who emphasize the morphology of the alternation of generations (MACMILLAN 20). Furthermore, it is to be noted that in the greater number of animals the gametophytic generation is omitted or perhaps to be considered as reduced to a single cell generation, and that here the conditions of maleness and femaleness are most essentially properties of individuals and structures that are wholly sporophytic.

It is clear that in the higher flowering plants maleness and femaleness are two series of morphological steps beginning in the development of stamens and pistils from cells of the closest somatic lineage. Any diploid or haploid nuclear organization can become either male or female according to whether its cell lineage leads through stamens or pistils. In this sense maleness and femaleness are acquired; they are conditions imposed upon cell organization rather than existing as separate inherent conditions; they begin in somatic differentiation that is fundamentally on the same basis as differentiation of stems, leaves, and sterile floral organs. Potentially maleness and femaleness (either morphological or physiological) reside in every cell of the sporophyte. The reduction divisions preceding the gametophytic divisions give the same range of nuclear organization to both kinds of spores.

It is such conditions, emphasized by the wide occurrence of hermaphrodites, that compel us to state the problems of sex determination in such questions as the following:

1. What physiological and chemical processes operate when sex differentiation appears and is initiated morphologically among organs which develop side by side from cells of the same somatic lineage?

2. Should we not regard dioecism as the suppression of maleness or femaleness in an individual as a whole (either in sporophytic or in gametophytic generations, or in both)?

We may note that intersexuality completely fills the gap between hermaphroditism and dioecism. In this respect the conditions in plants fully agree and supplement those reported in animals. Viewing all the evidence, we may at the present time make the following conclusions, which are in general harmony with the facts and the conclusions of GOLDSCHMIDT, BANTA, RIDDLE, and LILLIE: (1) Fundamentally maleness and femaleness reside in all somatic cells of all sporophytic individuals. (2) Maleness and femaleness are quantitative differentiations; there are all grades of intersexes. Maleness and femaleness are relative; there are all grades of compatibilities. (3) Sex determination, at least in hermaphrodites, is fundamentally a phenomenon of somatic differentiation that is ultimately associated with

processes of growth, development, and interaction of tissues, and subject to modification or even complete determination by them.

The older conception of mystical properties of maleness and femaleness have given place to what are fundamentally metabolic theories of sex determination. The principal points of difference in the large number of theories, thus to be grouped, lie in questions regarding (1) time of determination, (2) whether the two sexes are two contrasted conditions or simply phases of the same general property, (3) to what extent sex development in the individual is an evolution or an epigenesis, and (4) to what extent a physical basis can be related to differences in the amount of chromatin present.

To DARWIN and many of his contemporaries the evolutionary and adaptational significance of variations in sex were points of principal interest. That such variations fundamentally involve physiological processes operating in the organism was of course recognized. The increased femaleness seen in females of certain gynodioecious species was considered by DARWIN as involving the principle of compensation; with decreased expenditure of energy in development of male organs there was a greater supply for development and function of female organs. The doctrine of conservation in expenditure for useless organs was likewise applied to the tendency to gynomonoecism as seen in such a species as *P. lanceolata* (LUDWIG 19); the stamens in the uppermost flowers of a spike tend to be useless, and this was supposed to induce their elimination. The tendency to poor development of flowers at the tips of spikes, however, may be purely the result of food supply being diverted for use of lower flowers, and as such may be on quite a different physiological basis from the condition that makes an individual only female. The intimate association of many proterogynous flowers in a spike, however, may well give opportunity for changes in metabolic processes (RIDDLE) or influence of hormones (LILLIE).

A very interesting and suggestive conception which has frequently been proposed is embodied in the view that maleness is a "katabolic habit" of body (we may now add *of an organ*) induced by preponderance of waste over repair, and that femaleness is an

"anabolic habit" induced by conditions favoring constructive processes (GEDDES and THOMSON 10).

The physical basis for different metabolic activity is to be sought in qualitative or quantitative differences; the same kind of substance may be involved quantitatively or different substances may be involved either qualitatively or quantitatively.

The recent theory of the sex chromosome is in one aspect a metabolic theory in which different amounts of chromatin material in the nucleus may be considered as affording a physical basis for quantitative and perhaps qualitative metabolic differences. The theory fails as a broad biological law in not applying to the conditions of hermaphroditism as already discussed, and also in assuming that in dioecious species there is a determination of sex at the time of fertilization that is exclusive for the zygote. As intersexuality reveals, sex in zygotes of dioecious species is not necessarily irreversible (see especially RIDDLE and LILLIE); and experimental work has shown (see especially RIDDLE) that the distribution of sexes among the offspring may be controlled in a measure which breaks up the chromosomal correlation.

Most noteworthy of the more recent experimental data bearing on the chemical nature of sex determination are the results of RIDDLE. He has shown that in the pigeon "the male sex is an expression of metabolism at a higher level, the female sex of metabolism at a lower or more conservative level" (**22**, p. 322). The chemical nature of the eggs produced by a single female mated with a male is found to be subject to change according to whether egg production is forced or otherwise, and sex can thus be controlled. The physical basis for differences in metabolic activity is to be found in changes in the chemical organization and relations of the food substances. That such changes can readily occur is quite in harmony with well known facts as to the chemical differences in metabolic substances produced by an organ under different conditions. In the case of sex control in the pigeons it appears that it is not the amount of one or more kinds of food substances, but the different chemical nature of them, induced by the condition of the mother, that leads to differences in metabolism which determine the sex of the offspring.

The development of perfect flowers in hermaphrodites shows that male and female organs may originate side by side. That stamens and pistils exhibit differences in nutritive and metabolic activities is obvious, most marked of which perhaps is the temporary nature of the stamens and the more permanent and vegetative nature of the ovary portion of the pistil. The life processes of the two develop along somewhat different lines, as the structure and physiology of the respective spores, gametophytes, and sex cells fully indicate. Such organic specificity is well known frequently to involve specific differences in chemical organization. This, however, is not indicative that the essential nature of fertilization processes is dependent on such differences.

There seems to be no exception to the rule that in perfect flowers the male organs constitute an outer and lower whorl, the primary anlagen of which are laid down slightly ahead of those for the female. Such a general mode of development it would seem must have special significance in respect to sex differentiation. Such conditions, however, are adaptive both to immediate and to more remote function of the parts involved. When conditions in monoecious forms are reviewed it is to be noted that when grouped in spikes and catkins the staminate flowers are as a rule about the pistillate, either when both are in a same catkin or when they are in different catkins. Here, however, direct adaptations for facilitating pollination are in evidence.

The phenomena of intersexuality in plants and animals indicate clearly that neither hermaphroditism nor dioecism are fixed conditions for species or for individuals as such. Maleness and femaleness are subject to much lability; they are even reversible; the physical and chemical substances involved are subject to modification in ontogeny. The factors in sex determination for the individual as a whole or for individual sex organs are highly variable. Such conditions give support to a metabolic and epigenetic theory of sex in so far as the nature of sex is revealed in the morphological differentiation of sex organs.

NEW YORK BOTANICAL GARDEN
NEW YORK

LITERATURE CITED

1. BANTA, ARTHUR M., Sex intergrades in a species of Crustacea. Proc. Nat. Acad. Sci. 2:578–583. 1916.
2. BARTLETT, H. H., On gynodioecism in *Plantago lanceolata*. Rhodora 13:199–206. 1911.
3. ———, Inheritance of sex forms in *Plantago lanceolata*. Rhodora 15: 173–178. 1913.
4. CORRENS, C., Die Vererbung der geschlechtsformen bei den gynodioecischen Pflanzen. Ber. Deutsch. Bot. Gesells. 24:459–474. 1906.
5. ———, Zur Kenntnis der geschlechtsformen polygamer Blütenpflanzen und ihrer Beeinflussbarkeit. Jahrb. Wiss. Bot. 44:124–173. 1907.
6. DARWIN, CHARLES, On the sexual relations of the three forms of *Lythrum Salicaria*. Jour. Linn. Soc. 8:169–196. 1865.
7. ———, Forms of flowers. 1877.
8. DAVEY, A. J., and GIBSON, C. M., Note on the distribution of sexes in *Myrica Gale*. New Phytol. 16:147–151. 1917.
9. GATES, R. R., and GOODSPEED, T. H., Pollen sterility in relation to crossing. Science 43:859–861. 1916.
10. GEDDES, P., and THOMSON, J. A., The evolution of sex. 1889.
11. GOLDSCHMIDT, RICHARD, A preliminary report on further experiments in inheritance and determination of sex. Proc. Nat. Acad. Sci. 2:53–58. 1916.
12. ———, Experimental intersexuality and the sex-problem. Amer. Nat. 50:705–718. 1916.
13. ———, A further contribution to the theory of sex. Jour. Exp. Zoology 22:593–611. 1917.
14. JEFFREY, E. C., The mutation myth. Science 39:488–491. 1914.
15. ———, Spore conditions in hybrids and the mutation hypothesis of DE VRIES. BOT. GAZ. 58:322–336. 1914.
16. ———, Some fundamental morphological objections to the mutation theory of DE VRIES. Amer. Nat. 49:5–21. 1915.
17. LILLIE, FRANK R., Sex-determination and sex-differentiation in mammals. Proc. Nat. Acad. Sci. 3:464–470. 1917.
18. ———, The free-martin: a study of the action of sex-hormones in the foetal life of cattle. Jour. Exp. Zoology 23:371–452. 1917.
19. LUDWIG, F., Über die Blütenformen von *Plantago lanceolata* L. und die Erscheinung der Gynodiöcie. Bot. Centralbl. 1:331–333. 1880.
20. MACMILLAN, CONWAY, Proceedings Madison Botanical Congress. 1894 (p. 35).
21. PELLEW, CAROLINE, Types of segregation. Jour. Genetics 6:317–339. 1917.
22. RIDDLE, OSCAR, The control of the sex-ratio. Jour. Wash. Acad. Sci. 7:319–356. 1917.

STOUT on PLANTAGO

STOUT on PLANTAGO

23. RIDDLE, OSCAR, The theory of sex as stated in terms of results of studies on pigeons. Science N.S. 46:19–24. 1917.
24. SCHULZ, AUGUST, Beiträge zur Kenntniss der Bestäubungseinrichtungen und Geschlechtsvertheilung bei den Pflanzen. Bibl. Bot. 2: Heft 10, 1–103. 1888.
25. STOUT, A. B., Self- and cross-pollinations in *Cichorium Intybus* with reference to sterility. Mem. N.Y. Bot. Gard. 6:333–454. 1916.
26. STRASBURGER, E., Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung. 1884.

EXPLANATION OF PLATES XII, XIII

PLATE XII

Flowers ×2.5; stamens ×7.5; microspores ×110

FIGS. 1–3.—Flower, anther, and pollen of typical first form hermaphrodite.

FIGS. 4–6.—From typical second form hermaphrodite.

FIGS. 7–10.—Flowers, stamens, and petals of females of closed corolla type.

FIGS. 11–35.—From various intersexes each having flowers decidedly uniform.

FIGS. 36–48.—Illustrate various grades of intersexuality appearing among stamens and flowers produced by single individuals.

PLATE XIII

From photograph taken June 19, 1916, showing spikes at about one-half natural size.

FIG. 49.—Typical spike of first form hermaphrodite.

FIG. 50.—Spikes of plant no. 11; stamens differing slightly from those of first form hermaphrodite.

FIG. 51.—Spike of plant no. 37; all filaments short and of unequal lengths.

FIG. 52.—Spikes of typical second form hermaphrodite.

FIG. 53.—Three spikes from plant no. 39.

FIG. 54.—Spikes from plant no. 20; large non-dehiscent anthers conspicuous.

FIG. 55.—Spikes showing wide variation in character of stamens; many stamen forms present in flowers throughout entire spike; showing well reflexed corolla lobes.

FIG. 56.—Spikes of plant no. 46; flowers for drawings nos. 46, 47, and 48 taken from middle of spike at right; also showing elongated pistils as frequently developed.

FIG. 57.—Three spikes of various ages from female plant of closed corolla type; spike at right full of ripe seed.

SEXUALITY IN CUNNINGHAMELLA[1]

OWEN F. BURGER

The factors governing sexual reproduction in the Mucorineae have been regarded by various writers as due to the nutritive characters of the medium, the humidity of the surrounding atmosphere, the oxygen supply, and, lastly, the presence of conjugating male and female strains. So conflicting have been the theories that it is evident that the conditions controlling the production of zygospores are not so simple as many persons have supposed.

When EHRENBERG first discovered the zygospores of *Sporodinia grandis*, he regarded their formation as a process comparable to conjugation in *Spirogyra*. Since it was noticed that among the Mucorales zygosporic formation did not always occur, different workers gave their attention to the factors controlling their production. DEBARY, after working with *Rhizopus*, came to the conclusion that the lack of oxygen was the controlling factor. He found that the zygospores were produced in a closed tube more abundantly than in a tube opened to the air. VAN TIEGHEM repeated the work of DEBARY, and confirmed his views that desiccation could account for zygosporic formation in *Absidia septata*. On the other hand, he believed the zygospores described by BREFELD, in *Piptocephalis* and *Sporodinia*, were brought about by unfavorable food supply. BAINIER also thought that the environment influenced sexual reproduction, but he maintained that the formation of sexual organs was dependent on a nutritious rather than a poor substratum. ZOPF found *Pilobolus* producing zygospores, but ascribed their production to the fact that it was attacked by the parasites *Pleotrachelus fulgens* and *Syncephalis* sp. KLEBS maintained that their formation is induced by increased humidity, which hinders transpiration. FALCK, on the other hand, found that humidity and transpiration within normal limits have no effect on the production of zygospores. BREFELD maintained an agnostic attitude, and in a series of papers denied that

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 84.

the environments were, in themselves, sufficient to induce zygospore formation.

The early mycologists believed that all the genera were alike in their method of zygospore formation, but that their irregularity of occurrence was to be accounted for by one factor or a combination of the factors mentioned. BLAKESLEE, however, in 1904, showed that the Mucorineae could be divided into 2 classes, namely, homothallic and heterothallic forms, the homothallic forms being those whose zygospores are formed by the conjugation of gametes which are produced by hyphae of the same individual mycelium. To this group belong such genera as *Dicranophora*, *Sporodinia*, *Spinellus*, *Zygorynchus*, and some species of the genus *Mucor*. The heterothallic forms are those in which 2 kinds of individual mycelia occur. If the 2 individual strains of a heterothallic form are grown on the same medium, zygospores are produced at the point where the hyphae of the 2 strains meet. In this group belong most of the genera of the Mucorineae. Those 2 strains BLAKESLEE first called plus and minus, but in later papers he states that the sexes are as distinct as in higher organisms; the plus he calls female and the minus male. In the *Biological Bulletin* for August 1915 (p. 87) he says: "Conjugation in the Mucors is as definitely a sexual process as the morphologically more complex types of reproduction in higher forms, and the sexes seem even more sharply distinct." In the homothallic form *Zygorynchus heterogamus* one gamete is larger than the other; BLAKESLEE calls the larger gamete female and the smaller male. If the gametes are strictly male or female, as he maintains, there must have been a segregation of the male and the female nuclei in the respective gametes. In the heterothallic forms the male and female nuclei are segregated in different mycelia, and all the gametes produced by a single mycelium, therefore, must be either male or female.

According to BLAKESLEE, whenever a plus strain meets a minus strain of the same species, zygospores are produced. When opposite strains of different species meet, progametes only are formed, producing what he calls imperfect hybridization. No zygospores are produced; not even are gametes formed. The stimulus

from either strain is able to call forth the formation of progametes only in the other. Since he considers opposite strains to be zygotactic, he is thus able to find the sex of an unknown strain by growing it on a medium with a strain whose sex has previously been determined. Thus, if a strain whose sex is not known is grown on an agar plate with a plus strain, and if a sexual reaction is obtained, the new strain is considered to be minus. If no sexual reaction takes place, however, the strain is then contrasted with a minus strain; if sexual reaction is obtained, the new strain is considered plus; and if there is no sexual reaction with either the plus or minus strains, it is considered neutral. During his work BLAKESLEE found several strains of different species of Mucorineae which showed no reaction to either of his test species *Mucor V.* plus and minus. Those inactive strains he called neutrals, and believed them to be produced by the environment, because strains under artificial cultivation are sometimes found to lose their power of conjugation. HAGEM also found sexually inactive strains which he isolated from the soil. From 52 different strains of *Mucor* he found that 20 were minus, 3 were plus, and 29 were neutrals. If the Mucors are dioecious, as he and BLAKESLEE maintain, this seems to be a remarkably large percentage of neutrals to be found in a natural environment.

There are other conditions which may account for neutrals as well as the unfavorable environment. BURGEFF contrasted a plus and a minus strain of *Phycomyces nitens*, and from the sporangium of the germinating zygospore he obtained some spores which were plus, others which were minus, and a third kind which were neutral. The mycelium arising from the plus spores was also plus and produced plus spores. From the minus spores was obtained a minus mycelium, which in turn produced minus spores. On the other hand, from the neutral spores a mycelium was obtained which gave 3 kinds of spores, plus, minus, and neutral. In the neutral spores he believed the plus and minus nuclei to be of equal numbers. Thus the zygotactic stimulus of one kind of nuclei is counterbalanced by the nuclei of the opposite sex in the same hyphae.

In *Cunninghamella* there is exhibited a neutrality toward different strains which is unlike either of the neutral conditions

mentioned. The peculiarity shown by the different strains in their methods of conjugating with each other seems to indicate that the difference in sex in *Cunninghamella* is quantitative rather than qualitative. If one of the strains is taken as plus and all the others which conjugate with it as minus, there are strains which will conjugate with both plus and minus strains. On the other hand, there are, for example, strains (A) which will not show reaction with certain ones (B) but will in turn react with a third strain (C) which showed a sexual reaction with (B). The ability of one strain to show an individual selective power in conjugating with certain other strains is interpreted as evidence of a quantitative difference. Thus, if 2 strains are contrasted whose gametes are compatible a sexual reaction will take place. On the other hand, if 2 strains are contrasted whose gametes are incompatible no sexual reaction will take place.

Method

For two years I have been working with *Cunninghamella bertholletiae*. The cultures were obtained from decaying nuts of *Bertholletia excelsa*, together with cultures communicated to me by Dr. THAXTER, Dr. BLAKESLEE, and students in the Harvard Cryptogamic Laboratory. Authentic cultures of *C. bertholletiae* and *C. elegans* were obtained from Holland. The pure cultures used were obtained by transferring spores with a flamed needle directly from a single isolated head to a slant agar tube. Oatmeal agar was found to give the best results with these forms. The medium was made in the following manner: to every 1000 cc. of water was added 50 gm. of oatmeal. The mixture was steamed for 20 minutes, then strained through a triple thickness of cheesecloth, and 2 per cent of agar was then added. It was again autoclaved for 20 minutes at 15 lbs. pressure, after which it was tubed and sterilized.

The best results were obtained by placing the contrast series in battery jars, which were lined with moist filter paper and incubated at a temperature of 27° C. The contrast series were made by inoculating a Petri dish with 2 strains. It has been shown by BLAKESLEE that zygospores are formed more readily in moist

chambers kept at this temperature than under ordinary laboratory conditions. The tubes containing the pure cultures were treated in the same manner, but no zygospores appeared, showing that the cultures were pure and not a mixture of strains.

Cunninghamella has been regarded by BLAKESLEE as dioecious, and therefore no zygospores should appear unless both sexual strains are present. As both strains of *C. bertholletiae* had not yet been found, I contrasted all my cultures of this species with BLAKESLEE'S plus and minus strains of *C. echinulata*, hoping to secure imperfect hybridization, and thus, if possible, to determine the plus or minus nature of my strains. Many series of plates were made in which the cultures were contrasted with both strains of *C. echinulata*, but, although different media were used and the plates were subjected to different temperatures, I was unable to obtain imperfect hybridization in a single instance.

Both strains of BLAKESLEE'S *Mucor V.* were also used with a similar result. When *C. echinulata* plus and minus, as separated by BLAKESLEE, were contrasted with his *Mucor V.* plus and minus, imperfect hybridization took place. Moreover, when both the sexual strains of either of them, *Mucor V.* or *C. echinulata*, were themselves contrasted, the production of zygospores showed that the strains of these 2 test species had not lost their sexual vitality.

Experimental work

I have 34 different cultures of the Mucorineae which were contrasted with each other as indicated in table I. Cultures 1–26 inclusive are strains of *C. bertholletiae*, no. 21 being an authentic culture which was obtained from Holland. Cultures 27–31 are strains of *C. echinulata*, and of these 27 and 28 are *C. echinulata*, plus and minus as separated by BLAKESLEE. Cultures 32 and 33 are *Mucor V.* minus and plus as separated by BLAKESLEE, and 34 is an authentic culture of *C. elegans* which was also obtained from BLAKESLEE.

CONTRAST SERIES A

Having been unable to get any sexual reaction by contrasting the different strains of *C. bertholletiae* with *C. echinulata* plus and minus, I contrasted *C. elegans* no. 34 with all my other cultures.

TABLE I

SEXUALITY IN *Cunninghamella*

CONTRAST SERIES	C. BERTHOLLETIAE																									C. echinulata					Mucor V.		C. elegans
Cultures	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Series A																																		
Culture 34	...	...	1	...	...	...	...	1	...	...	...	1	1	...	...	...	...	...	...	...	1	...	...	1	1	1	...	...	...	...	...	1	...	...
Series B																																		
Culture 21	×	×	×	...	...	...	×	...	×	×	...	...	×	×	...	×	×	...	...	×	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Series C																																		
Culture 9	...	...	×	×	×	×	×	×	...	...	...	×	×	...	×	...	...	...	...	×	×	...	...	...	...	...	...	...	...	...	...	...	...	...
Series D																																		
Culture 14	...	...	×	×	...	...	×	×	...	...	...	×	×	...	...	×	...	...	...	×	×	...	...	...	...	...	...	...	...	...	...	...	...	...
Series E																																		
Culture 7	...	...	...	...	...	...	...	...	×	×	...	...	...	×	...	×	×	...	...	×	×	...	...	...	...	...	...	...	...	...	...	...	...	...
Series F																																		
Culture 3	...	...	...	...	...	...	...	...	×	×	×	...	...	×	...	×	×	...	...	×	×	...	...	...	...	...	...	...	...	...	...	...	...	1
Series G																																		
Culture 10	...	...	×	...	...	...	×	×	...	...	...	×	×	...	...	...	...	×	×	...	×	×	×	...	...	...	...	...	...	...	...	...	...	...

As a result, imperfect hybridization took place between it and nos. 3, 8, 12, 13, 21, 24–26, and 32. Culture 25 is BLAKESLEE'S *Mucor V.* minus, therefore the culture of *C. elegans* with which it reacted would be plus. It also reacted with culture 21, which is the authentic culture of *C. bertholletiae.* Since I was unable to get a sexual reaction with the different strains of *C. bertholletiae* when contrasted directly with *Mucor V.* plus and minus, I had to determine their sexual character by this indirect method. Since, therefore, *C. elegans* no. 34, because of its reaction with *Mucor V.* minus, would necessarily be plus, according to BLAKESLEE, *C. bertholletiae* no. 21 would be minus since it reacted with *C. elegans* no. 34. On the other hand, the cultures which did not react with no. 34 would be regarded as either plus or neutral.

CONTRAST SERIES B

Since I had thus determined that the sex of no. 21, the authentic culture of *C. bertholletiae,* was minus, I then contrasted it with all my cultures of this species, and normal zygospores were formed with nos. 1, 2, 3, 7, 9, 10, 13, 14, 16, 17, and 20, while imperfect hybridization took place again with no. 34, the authentic culture of *C. elegans.* All of these, therefore, on a basis of BLAKESLEE'S theory, should be regarded as certainly plus. In contrast series A, however, it was proved that cultures 3 and 13, like no. 21, were minus, but in the present series B they both formed normal zygospores when contrasted with no. 21. If we assume, therefore, that the species is dioecious, it is impossible on the theory of heterothallism to account for the production of normal zygospores in series B as a result of the interaction on no. 21 with mycelia which had been demonstrated to belong to the same sex.

CONTRAST SERIES C

As we have seen, cultures of *C. bertholletiae* no. 9 formed zygospores in series B with no. 21, which in series A was shown to be minus. *C. bertholletiae* no. 9, therefore, according to the theory of BLAKESLEE, should be regarded as plus. This plus strain no. 9 was then contrasted with all the other cultures of *C. bertholletiae,* and as a result formed zygospores with nos. 3–8, 12, 13, 15, 20, and 21. This presumably plus strain, therefore, formed zygo-

spores with nos. 3, 13, and 20, all 3 of which also formed zygospores in series B with no. 21, which in series A was shown to be minus. Since then, as determined by contrast with no. 21 in series B, nos. 3, 9, 13, and 20 are all plus, we have the anomaly of a plus strain no. 9 conjugating with 3 other plus strains, nos. 3, 13, and 20.

If we assume, as shown in series A and series B, that nos. 21 and 9 are minus and plus respectively, cultures 1, 2, 3, 7, 9, 10, 13, 14, 16, 17, and 20, since they react with no. 21, would be minus, and nos. 3–8, 12, 13, 15, 20, and 21, since they react with no. 9, would be plus. The other cultures, 22–33, which did not conjugate with either 21 or 9, must, according to BLAKESLEE, be considered as neutrals or as belonging to a different species. Cultures 3, 7, 13, and 20, on the other hand, must be hermaphroditic, or sexually bivalent, since they are able to conjugate with both plus and minus strains.

CONTRAST SERIES D

In this series culture 14, which was shown to be plus in series B, was contrasted with all the other cultures of *C. bertholletiae* and formed zygospores with nos. 3, 4, 7, 8, 12, 13, 16, 20, and 21. In series B cultures 9 and 14 were both shown to be plus. If these 2 cultures are plus, that is, if they are of the same sex, they should behave in the same manner when contrasted with all the other cultures. As a matter of fact, however, we see that no. 9 formed zygospores in series C with nos. 3–8, 12, 13, 15, 20, and 21, while in the present series culture 14 showed no sexual reaction with cultures 5, 6, and 15, but formed zygospores with 16. Since culture 9 does not form zygospores with the same cultures as no. 14, they cannot be regarded, therefore, as sexually identical.

CONTRAST SERIES E

Culture no. 7 was next used and formed zygospores with nos. 9, 10, 14, 16, 17, 20, and 21. This culture was shown to be plus in series B when contrasted with no. 21, and here again we notice a selective power, the reactions of this plus strain not conforming in all respects to the 2 plus strains employed in contrast series C and D.

CONTRAST SERIES F

In this series culture no. 3, which was shown to be plus in series B, was contrasted as in the previous series with all the remaining numbers and formed zygospores with nos. 9–11, 14, 16, 17, 20, and 21. Culture no. 11, which up to this time had failed to react with plus strains and had been considered neutral, formed normal zygospores when contrasted with no. 3. The conditions were the same as those of the previous experiments so far as it was possible to duplicate them. This series, as well as the others, was repeated several times, and in no instance did no. 11 form zygospores with 9, 14, or 21, the plus strains used in series B, C, and D.

CONTRAST SERIES G

Culture no. 10, which was determined to be plus in series B when contrasted on agar plates with the different strains, formed zygospores with nos. 3, 7, 8, 12, 13, 18, 19, 21–23. Cultures 18, 19, 22, and 23 had not formed zygospores with any of the other strains of *C. bertholletiae* previously tested, and therefore were considered as neutral. Since, however, in this series they formed zygospores with no. 10, they must be assumed to be minus. The question here arises, why if they are minus have they not conjugated with cultures nos. 3, 7, 9, and 14, which were all proved to be plus by the contrasts made in series B?

As had previously been stated, the cultures used in these 4 series were obtained from transfers made by touching single heads with a sterile needle and transferring directly, but in order to preclude the possibility of a mixture of strains, on January 3, 1917, single-spore cultures were made from cultures 3, 9, and 21, which were selected from the critical numbers used in the contrast series described. These cultures were made by the poured plate method. The germinating spores were located with the compound microscope, and were picked out by means of a fine needle and transferred to culture tubes. I obtained 3 single spore cultures of no. 3, 2 cultures of no. 9, and 4 cultures of no. 21. On January 27, 1917, the following contrast cultures were made with these single spore mycelia.

CONTRAST SERIES H

Each of the single-spore cultures of no. 3 were contrasted with each of the 2 pure cultures of no. 9, and in every case normal zygospores were produced, although both nos. 3 and 9 were shown in series B to be plus.

CONTRAST SERIES I

Each of the 3 sub-cultures of no. 3 were contrasted with each of the 4 cultures of no. 21, and every contrast plate produced normal zygospores, although the latter had been shown to be minus in series A.

CONTRAST SERIES J

The 2 sub-cultures of no. 9, which was shown to be plus in series B, were contrasted with each of the 4 sub-cultures of no. 21, and each contrast plate produced normal zygospores. It is demonstrated, therefore, beyond question that cultures 3, 9, and 21 form zygospores with both plus and minus strains, and therefore are sexually bivalent or hermaphroditic. Single-spore cultures were made from these 3 strains to eliminate the objection that the cultures had been mixed. Each of the cultures, obtained from a single spore, from either strain formed zygospores with the single-spore cultures of the other 2 strains, thereby confirming the results of the previous contrast series.

In the above contrast series, nos. 1 and 2 have always remained constantly plus, while nos. 4–6, 12, 15, 18, 19, 22–26 were always minus. Nos. 3, 7–11, 13, 14, 16, 17, 20, 21, and 34, however, have reacted with both the so-called plus and minus strains. Those numbers which showed no sexual reaction with any of the strains of *C. bertholletiae* are nos. 27–33, and therefore, according to the accepted terminology, are neutrals. We must remember, however, that it was pointed out before that cultures 27 and 28 are *C. echinulata* plus and minus as separated by BLAKESLEE. These 2 cultures always formed normal zygospores when contrasted with each other, and therefore are not neutrals. Cultures 32 and 33 are *Mucor V.* minus and plus as separated by BLAKESLEE; these cultures also formed normal zygospores when contrasted with

each other, and therefore are not considered as neutrals. Whenever cultures 25 and 32 were contrasted with culture 34, which is *C. elegans*, imperfect hybridization took place. It must also be remembered that cultures 25 and 32 are minus, as separated by BLAKESLEE. It is peculiar that the plus strains of *C. echinulata* and *Mucor V.* showed no sexual reaction with any of the cultures of *C. bertholletiae*.

Discussion

During the entire work, although careful search for them was made, no zygospores were obtained in the pure cultures, a fact which indicates that this species is not homothallic. Since the strains show a selective power in conjugating with other strains, however, it is not a heterothallic form as defined by BLAKESLEE.

Since it was impossible to get any of the strains of *C. bertholletiae* to react with *C. echinulata* or *Mucor V.* as separated by BLAKESLEE, I had to use the indirect method to determine the sex of the different strains. In contrast series A, no. 25, which is *Mucor V.* minus, showed a sexual reaction with no. 34, which is an authentic culture of *C. elegans*. All the cultures which did not react with no. 34 must therefore be considered either of the same sex, which is minus or neutral.

In contrast series B culture no. 21 formed normal zygospores with cultures 3 and 13, which themselves were proved in contrast series A to be of the same sex as 21.

On the assumption that the species is dioecious, it is impossible to account for the production of normal zygospores as a result of the interaction of mycelia of the same "sex." The situation is further complicated by the behavior of some of the so-called "neutral strains."

On the basis of contrast series B, nos. 1, 2, 3, 7, 9, 10, 13, 14, 16, 17, and 20 are plus. On the other hand, in contrast series A nos. 3, 8, 12, 13, 21, 24–26, and 32 were shown to be minus. According to BLAKESLEE'S views, nos. 4–6, 11, 15, 18, 19, 22, 23, 27, 28, 30, 31, and 33 must be neutral, for they show no sexual reaction with 2 strains which were shown to be plus and minus respectively.

The "neutrals" 4–6 and 15, however, formed zygospores when contrasted with culture no. 9 in series C. Since 9 was proved in series B to be plus, nos. 4–6 and 15 would therefore be considered as minus.

The neutral no. 11 formed normal zygospores when contrasted with 3 in series F. We have shown, however, that 3 is plus in series B and minus in series A; therefore no. 3 would have to be considered hermaphroditic.

Nos. 18, 19, 22, and 23, which were shown to be neutrals, formed normal zygospores when contrasted with no. 10 in series G. No. 10 was shown to be plus in series B; therefore nos. 18, 19, 22, and 23 must be considered as minus. If these numbers are considered as minus, the question arises, why did they not conjugate with nos. 34, 21, 9, 7, and 3, which were all shown to be plus?

The evidence that nos. 4–6, 11, 15, 18, 19, 22, 23, 27, 28, 30, 31, and 33 are "neutral" does not seem to fit the accepted conception of this term, for they are able to form normal zygospores when contrasted with the strains whose gametes are compatible.

If plus and minus strains of the *Mucor V.* are female and male respectively, they should have shown a sexual reaction with different strains of *C. bertholletiae* which were capable of forming zygospores when contrasted with each other. This neutrality cannot be explained on the grounds of loss of vitality; neither do I believe this neutrality can be explained by BURGEFF'S hypothesis, that there are the same number of plus and minus nuclei in a given hypha, the plus nuclei annulling the zygotactic influence of the minus. It has been shown that a strain which is neutral to 2 strains, which themselves are plus and minus respectively, has the power to conjugate with a third strain if their gametes are compatible. The writer is unable at the present time to give any satisfactory explanation of this pseudo-heterothallic condition in *Cunninghamella bertholletiae*, but it is evident that we still have much to learn as to the sexual conditions in the Mucorineae, especially in relation to so-called neutral strains.

Summary

1. In *Cunninghamella* there does not exist sexual dimorphism.

2. *C. echinulata* plus and minus, or *Mucor V.* plus and minus as separated by BLAKESLEE, are unable to form progametes or gametes when contrasted with any one of 26 cultures of *C. bertholletiae.*

3. Many of these cultures of *C. bertholletiae* were able to form zygospores when contrasted with certain other cultures of this same species.

4. There exists a selective power in some strains to form zygospores with certain other strains. This condition of pseudo-heterothallism cannot be explained at present.

5. There exists a condition in some strains which might be called hermaphroditism.

6. In none of the hermaphroditic strains did branches of the same hyphae conjugate.

7. Zygospores were produced only when 2 strains were contracted whose gametes were compatible.

I wish to express my gratitude to Professor THAXTER, under whose direction the work was undertaken, also to Dr. BLAKESLEE and Mr. A. R. BUTLER for various cultures used in this study.

HARVARD UNIVERSITY
CAMBRIDGE, MASS.

BRIEFER ARTICLES

ERRORS IN DOUBLE NOMENCLATURE

In naming plants having so few well marked individual features as the micro-fungi, it is natural that the appellations should often be taken from the character of the substratum. Among the Uredinales names are frequently derived from the hosts on which they are found; thus *Aecidium Aesculi* is so named because it grows or was believed to grow on *Aesculus*. Many collections of rusts include simply the leaves of the host, or only fragments of leaves or of other parts. Mycologists generally depend upon the collector or some phanerogamic specialist to supply the host determination. The critical taxonomist, however, must be on the alert to detect anything that might possibly invalidate the name applied to the host, as well as to make sure of the correctness of the name given to the parasite. Thus it comes about that the taxonomic uredinologist must deal in double nomenclature, that pertaining both to the host and to the fungus. To misapprehend the identity of either the host or of its unbidden guest may entail deplorable consequences.

The object of this note is to point out a curious error of this sort, which went through the hands of more than half a dozen able taxonomists undetected before passing into print. In the account of the rusts collected by Dr. and Mrs. J. N. Rose in the Andes, given by the writer in the Botanical Gazette for May 1918 (p. 470), 2 new species are proposed, both on Solanaceous hosts. One is *Puccinia Nicotianae* on a species of *Nicotiana*, and the other following is *P. Acnisti* on a species of *Acnistus*. If the descriptions of these 2 species be compared, they will be found to be remarkably similar. In fact, the only important differences are that the first gives measurements for more globoid urediniospores, and makes the wall of the teliospore slightly thicker above and verrucose instead of smooth, as compared with the second. These descriptions were drawn up independently by different workers, and were not closely compared until taken in hand by a third investigator after they were published, who undertook to fit them into a general key. Upon reexamining the specimens these differences vanish. The variation in length of urediniospores is greater than the first description states, the teliospores are not really thicker above, and their surface is obscurely verrucose, although sometimes seemingly smooth.

Placing the collections side by side, they were found to look alike, both consisting of a few large leaves well covered with rust. It will be noticed that the published data for the hosts are identical, even to the number, in fact having originally consisted of a single collection, which was separated by the collectors into two parts, one part being distinguished from the other by adding the letter "a" to the number. The material was handled at the herbarium of the National Museum, and one of the two parts was examined also at the Gray Herbarium. Thus at least three highly trained taxonomists passed upon the identity of the hosts, or rather the host, and the three microscopists of the Purdue Agricultural Experiment Station, who passed upon the identity of the rusts, or rather the rust, did their part without a suspicion of anything amiss. It took a seventh man to bring the two descriptions and the sets of material together and point out that only one rust and one host were involved. The two packets of material were subsequently sent to Mr. PAUL C. STANDLEY of the National Museum with a statement of the situation, and were returned with the information that the host was neither *Nicotiana tomentosa* nor *Acnistus arborescens* as published, but was *Acnistus aggregatus* (R. and P.) Miers.

It would have been unnecessary to give a detailed account of this series of errors had the duplicate names of the rust in their fortuitous position on the page been reversed. The American Code of Nomenclature recognizes page position in deciding priority. In this case, however, it may be assumed that duplicate names having been given simultaneously to the same fungus, or as near as it is possible to do so in print, one of them correctly formed and the other glaringly erroneous, the correct name should be maintained and the other treated as a blunder and discarded. This disposition of the case is also in accord with the International Rules of Nomenclature, which give to the author the privilege of choosing between two names of the same date, which subsequently he considers to be conspecific (Article 46). The correct name to include both descriptions, as well as other data, therefore, is *Puccinia Acnisti* Arth., on *Acnistus aggregatus*. Of course this instance has no bearing upon such inappropriate but tenable names as *Puccinia Distichlidis*, at first supposed to be a rust on *Distichlis spicata*, but years later found to be on *Spartina gracilis*, or as *P. Sorghi*, now known never to occur on *Sorghum*.

The same species of fungus, to which this note refers, has been listed in the account of the Uredinales of Costa Rica, where it is correctly given as *Puccinia Acnisti*, and in this case is on *Acnistus arborescens* (Mycologia 10:138).—J. C. ARTHUR, *Purdue University, Lafayette, Ind.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Distribution of Pinus Banksiana and Thuja occidentalis.—In a recent issue of this journal HUTCHINSON[1] has discussed the limiting factors controlling the distribution of forest trees in northern Canada. Most of his points seem to have been well taken, and his conclusions in accord with the observed facts. He lays considerable emphasis on certain peculiarities in the distribution of *Pinus Banksiana* and *Thuja occidentalis*. The latter he regards as having migrated from a limited central area so slowly that it has not reached its ecological limits; while the former has had its extent modified by competition, which it seems less able to resist than severe conditions in its environment.

More recently FERNALD[2] has offered another explanation for the irregularities of these two trees, and has criticized HUTCHINSON'S article in a decidedly unsympathetic manner. He comments upon the accidental use of *Abies canadensis* in the legend of a map when it is quite evident from the text that *Abies balsamea* is intended (this correction was made by the author in the errata published in the June number of the BOTANICAL GAZETTE). He also points out certain minor omissions and irregularities which somewhat modify HUTCHINSON'S limits of various species. His main point, however, is to offer an entirely different explanation for the peculiarities in the range of the two trees just mentioned. From data obtained chiefly from the reports of the Geological Survey of Canada, he shows that *Pinus Banksiana* is found principally on sands, acid rocks, and in acid swamps. This seems to support his contention that the "Banksiana pine is a pronounced oxylophyte." The evidence presented is such that it seems at least entirely probable that the limiting factor in the distribution of this tree may be largely one of soil. This is rather strengthened by the records of certain of its outposts in southeastern Minnesota[3] upon sandy soil. It does not seem, however, that the fact that *Pinus Banksiana* is to be regarded as a tree of acid habitats invalidates HUTCHINSON'S conclusion that it is limited in many parts of its range by competition.

FERNALD seems also to make a good case for *Thuja* being confined in its best development and in many of its outlying stations to calcareous areas. The failure of *Thuja* to reach Newfoundland would be due, as he contends, to

[1] BOT. GAZ. 66:465–493. 1919.

[2] FERNALD, M. L., Lithological factors limiting the ranges of *Pinus Banksiana* and *Thuja occidentalis*. Rhodora 21:41–67. 1919.

[3] ROSENDAHL, C. O., and BUTTERS, F. K., On the occurrence of *Pinus Banksiana* in southeastern Minnesota. Plant World 21:107–113. 1918.

the barrier of siliceous rock upon the adjacent mainland. His view that "the Canadian cedar swamp is, then, a phase of WARMING'S calcareous low-moore" appears less probable, and does not seem to explain the frequent presence of *Thuja* in associations with *Larix* in bogs.

It would seem to remain for some one possessing intimate knowledge of the northern forests, but without prejudice for or against the chemical theory of soil control, to harmonize such opposing views as those of HUTCHINSON and FERNALD, by showing that each contributes to the solution of a complex problem, and that the truth lies at neither extreme.—GEO. D. FULLER.

Hybrid vigor.—This subject is brought up to date and ably discussed by JONES[4] in the publication of his latest experiments with corn. The author has continued the inbreeding experiments started by EAST and HAYES. As was predicted, the inbred strains have now reached a condition of almost complete homozygosity, so that further inbreeding no longer brings decrease in vigor, and crossing within the strain brings no increase. The author amplifies somewhat his previously published[5] interpretation of hybrid vigor on the basis of dominance of linked factors.

In addition to the main thesis, some very interesting by-products are discussed. As a practical method of utilizing hybrid vigor in corn, SHULL[6] and others have advised isolating strains A and B and using for seed corn every generation the F_1 grains produced by $A \times B$. A disadvantage of this method lies in the fact that these seeds are usually small, for, although they contain an F_1 embryo, the amount of endosperm is that of the maternal parent (from an inbred, "non-vigorous" race, A or B). Since these seeds are small, the F_1 individuals get a poor start, limiting their expression of hybrid vigor. To overcome this difficulty JONES proposes an intelligent use of 4 selected strains thus: $A \times B$ giving AB; $C \times D$ giving CD; $AB \times CD$ giving the seed corn to be used, which will have sufficient endosperm for a good start and will display hybrid vigor as well.

Carrying further his experiment[7] with mixed foreign and own pollen ("yellow" and "white" pollen), JONES attempted to discover whether there was any selective fertilization in favor of the foreign pollen; this might have been expected from the advantages which foreign pollen brought, as well as from the well-known behavior in self-sterile races. The results, however, pointed consistently in the opposite direction; own pollen was slightly but

---

[4] JONES, D. F., The effects of breeding and cross-breeding upon development. Conn. Exper. Sta. Bull. 207. pp. 100. *pls.* 12. 1918.

[5] BOT. GAZ. 56:70–72. 1918.

[6] SHULL, G. H., Hybridization methods in corn breeding. Amer. Breeders Mag. 1:98–107. 1910.

[7] JONES, D. F., Bearing of heterosis upon double fertilization. BOT. GAZ. 65:324–333. *figs.* 3. 1918.

regularly more successful than foreign pollen. "If this is true, crossing is without effect until . . . . the union of the male and female nuclei." In certain of the inbred strains the author records a marked tendency toward dioecism. Some of the inbred strains which maintained the highest ovule development were the most deficient in pollen development, while the exact reverse was true in other strains.

In his general discussion the author suggests that the advantages of hybrid vigor may have played their part in the rise of the sporophyte generation. Certainly if his interpretation of the phenomenon is correct (the reviewer believes it is), the advantages of hybrid vigor would be impossible in the gametophyte generation with its haploid equipment.—MERLE C. COULTER.

Perennating fruit of Cactaceae.—JOHNSON[8] has investigated the remarkable behavior of the fruits of certain Cactaceae, using *Opuntia fulgida* as material. The fruits of these Cactaceae remain attached to the plant and actively growing for several or many years. The fruit of *O. fulgida* not only remains attached, unripened, and steadily growing, but the seeds are never shed from the fruit. In addition to this, the matured fruit, or even the ovary of the unripened flower, may give rise to secondary flowers and so to other fruits. As many as 4 or 5 generations of flowers and fruits may thus be formed in a single season. If a mature fruit falls on moist soil, it may develop adventitious roots and shoots and thus initiate a new plant.

The early development of the ovary resembles that of a young vegetative joint, and is entirely stemlike in appearance, with its evanescent leaves, tubercles, and axillary areoles. It is evident, for many reasons, that the whole outer wall of the ovary and fruit is morphologically of stem origin. The continuous formation of flowers is remarkable, as indicated by the following description: "From the axillary buds, or areoles, of the primary flowers that open in May, arise secondary flowers which open in June. From areoles of these, in turn, tertiary flowers open in July, and on the latter quaternary flowers bloom forth in August."

The contribution contains much interesting material that cannot be included in a brief review, but it all presents the unusual habits of a remarkable group of plants.—J. M. C.

Alaria.—YENDO[9] has published a monograph of *Alaria* which is remarkably full in its details and noteworthy in the quality of its plates. The introductory pages deal with the morphology of the genus, every region of the plant being considered, and the development and life history presented, so that the

---

[8] JOHNSON, DUNCAN S., The fruit of *Opuntia fulgida*. A study of perennation and proliferation in the fruits of certain Cactaceae. Publ. Carnegie Inst. pp. 62. *pls. 12.* 1918.

[9] YENDO, KICHISABURO, A monograph of the genus *Alaria*. Jour. Coll. Sci. Univ. Tokyo 43:1–145. *pls. 19.* 1919.

result is a valuable contribution to the morphology of the Laminariaceae. The distribution and habitat are also presented in full, as well as an interesting account of the economic use of the genus. More than 32 species have been described since the genus was established in 1830, but the author recognizes only 15. The uncertainty of specific limitations has been due to the fact that the describer has not observed the stages of development or the effects of different habitats, so that different forms of one species have been described as distinct species. The author has studied *Alaria* in its habitats throughout the northern Pacific from Vancouver Island to Japan, and the result is a reorganized presentation of the genus, only one new species being recognized (*A. ochotensis*), but a number of old "species" disappearing as stages or habitat forms of other species.—J. M. C.

Cones of Williamsonia.—The organization of the cones of *Williamsonia gigas* has been a "palaeobotanical puzzle" ever since the original description in 1849. Since that date it is said that approximately 100 memoirs have discussed this subject. The late E. A. NEWELL ARBER[10] left a brief paper summing up the difficulties, and suggesting conclusions. The difficulties presented are 4 in number: (1) were the cones monosporangiate or bisporangiate? (2) where were the microsporophylls attached? (3) what structure was borne on the axis of the cone above the megasporophylls? (4) was there an infundibular expansion, similar in form to the united whorl of microsporophylls, but sterile, and where was it attached?

The answers given are as follows: (1) the cones were probably monosporangiate; (2) the ovulate cone bore only seeds and interseminal scales on a conical axis; (3) the staminate cone had an urn-shaped axis, sheathed below, which bore apically a whorl of partly united microsporophylls and no interseminal scales; (4) there is no evidence of any sterile infundibular organ attached to or terminating either cone.—J. M. C.

Zingiberaceae of Java.—In 1904 VALETON[11] published an account of the Zingiberaceae of Java. This account he has now supplemented[12] by further investigation during the last 15 years. The present extensive paper is only the first part, dealing chiefly with *Curcuma*, *Gastrochilus*, *Kaempferia*, and *Zingiber*. There is a very full discussion of the characters of the family, and each species is presented in great detail. The 4 genera referred to are represented as follows: *Curcuma*, 21 spp. (10 new); *Gastrochilus*, 16 spp. (5 new); *Kaempferia*, 4 spp.; *Zingiber*, 17 spp. (5 new).—J. M. C.

---

[10] ARBER, E. A. NEWELL, Remarks on the organization of the cones of *Williamsonia gigas*. Ann. Botany **33**:173–179. *figs.* 5. 1919.

[11] Bull. Inst. Bot. Buitenzorg. no. 20. 1904.

[12] VALETON, TH., New notes on the Zingiberaceae of Java and Malaya. Bull. Jard. Bot. Buitenzorg. no. 27. pp. 168. *pls.* 30. 1918.

PLANT GENETICS

By JOHN M. COULTER

Head of the Department of Botany in the University of Chicago

and MERLE M. COULTER

Instructor in Plant Genetics in the University of Chicago

¶ This book has been written to meet an increasing need among botanical students. Such students in these days, in whatever phase of botany they may be specializing, find it necessary to read with understanding much of the literature of plant genetics, because it is becoming increasingly significant in all botanical problems. This means that teachers and investigators must be able to command the literature of plant genetics, much of which has been so complex as to be a closed book for the uninitiated. *Plant Genetics* is an attempt to open this subject to botanical students.

¶ The book is not intended to be a thorough, authoritative text, but a relatively simple presentation of the more significant investigations on plant genetics which will initiate the student into the subject. Material dealing with some highly specialized phases of genetics and material that is very complex has been purposely omitted for pedagogical reasons. In short, the book is an easy introduction to plant genetics.

x+214 pages, 12mo, cloth; $1.50 (postpaid $1.65)

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO - - - - - - ILLINOIS

A SOURCE BOOK OF

BIOLOGICAL NATURE-STUDY

By ELLIOT R. DOWNING

Associate Professor of Natural Science in the School of Education
The University of Chicago

For teacher-training classes, school libraries, and general reading by everyone who is interested in the common animals and plants. The book is illustrated with more than three hundred sketches and photographs, and the descriptions and keys further facilitate the identification of plant and animal forms. The author gives suggestive discussions of the life-histories and the problems that confront animals and plants, and includes some discussion of their economic value and their conservation.

Octavo size, cloth; $3.00, postpaid $3.15

The *Source Book* is the second published volume of

The University of Chicago Nature-Study Series

The first volume is

A Field and Laboratory Guide in Biological Nature-Study

Loose leaf; $1.00, postpaid $1.10

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO - - - - - - ILLINOIS

It Gives the Typist More Time to Type

These three things—***inserting the paper, typing, line spacing,*** are all the typist has to do when she operates the

SELF-STARTING REMINGTON

Hand settings of the carriage, which are necessary on all other typewriters, are eliminated absolutely—and that means a time saving of 15 to 25 per cent in transcribing business letters. The Remington Self-Starter, or automatic indenter, gives the typist more time to type. That means a bigger day's work with no more effort; bigger typewriter value at no greater price.

REMINGTON TYPEWRITER COMPANY
(Incorporated)
37 Broadway New York

FINE INKS AND ADHESIVES
For those who KNOW

Higgins'

- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

At Dealers Generally

CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street Brooklyn, N.Y.

Combined Balopticon
For Lantern Slides and Opaque Objects
Price $150.00

Bausch and Lomb
BALOPTICON
THE PERFECT STEREOPTICON

for every practical form of optical projection is again in the market, backed by increased facilities which have been occupied in war service of a most significant character.

Our release from this service is of course gradual. As the demands made upon us by the government are lessened from month to month, however, we are enabled to increase deliveries to our other patrons.

Write for Balopticon catalog, with price list of our revised line, and inform us regarding your requirements.

Bausch & Lomb Optical Co.
554 ST. PAUL STREET, ROCHESTER, N.Y.
NEW YORK WASHINGTON CHICAGO SAN FRANCISCO

Leading American Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

Volume LXVIII Number 3

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

SEPTEMBER 1919

Phytogeography of the Eastern Mountain-Front in Colorado. I. Physical Geography and Distribution of Vegetation. Contributions from the Hull Botanical Laboratory 251 - - - - Arthur G. Vestal 153
(With seventeen figures)

On Nitrification. III. The Isolation and Description of the Nitrite Ferment - - - - - - - - Augusto Bonazzi 194
(With Plate XIV)

Polyxylic Stem of Cycas media. Contributions from the Hull Botanical Laboratory 252 - - - - - - - Ward L. Miller 208
(With eleven figures)

A Parasite of the Tree Fern (Cyathea) - F. L. Stevens and Nora Dalbey 222
(With Plates XV, XVI)

Anatomy of Lycopodium reflexum - - - - - J. Ben Hill 226
(With five figures)

Current Literature
Notes for Students - - - - - - - - - - 232

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London and Edinburgh*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Volume LXVIII Number 3

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued September 16, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, £1 12*s.* 6*d.* each; single copies, including postage, 3*s.* 6*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 18 Peking Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

VOLUME LXVIII NUMBER 3

THE
BOTANICAL GAZETTE

SEPTEMBER 1919

PHYTOGEOGRAPHY OF THE EASTERN MOUNTAIN-FRONT IN COLORADO

I. PHYSICAL GEOGRAPHY AND DISTRIBUTION OF VEGETATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 251

ARTHUR G. VESTAL

(WITH SEVENTEEN FIGURES)

Introduction

The plant geography of a region is the effect of the working of present and former environmental influences upon the floras and vegetation-complexes which exist and have existed within the region and in the regions adjoining. The region of present study, lying as it does in the transition belt between two great geographic divisions of North America, the Great Plains, or western part of the prairie region, and the Rocky Mountains, has some of the characters of both; others of its physical and vegetational features are transitional, intermediate; and it has certain peculiarities, differing thus from the regions on either side. Since climatic variation, differences of soil and of topography, and multiformity of vegetation-types are considerable, the plant-covering of the area is a complex of many diverse types. Descriptive accounts of the plant associations of plains and foothills have already been published (17, 18), so that the present article may deal more particularly with geographic description and geographic relations.

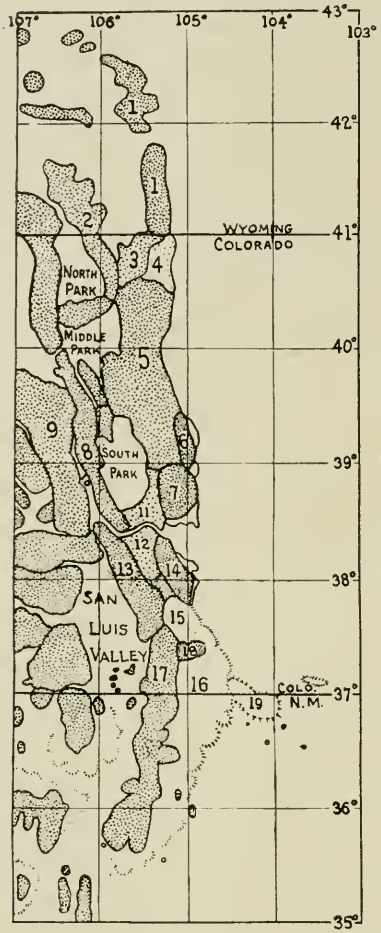


FIG. 1.—Map of southern Rocky Mountains, except westernmost ranges; mountain areas shaded; names of areas indicated by numbers are: 1, Laramie Mountains; 2, Medicine Bow Range; 3, low mountain area connecting Laramie and Front ranges; 4, foothills of Poudre River area; 5, Front Range; 6, Rampart Range; 7, Pike's Peak highland; 8, Park Range; 9, Saguache Range; 10, Upper Arkansas Valley (between 9 and 8); 11, low mountains; 12, Wet Mountain Valley; 13, Sangre de Cristo Range; 14, Wet and Greenhorn mountains; 15, Huerfano Park; 16, southern sedimentary plateau; 17, Culebra Range; 18, Spanish Peaks highland; 19, Raton mesas.

While in general the plains and mountains contrast rather sharply at their junction, this is not always true; the mountain-front is a transition zone in places a number of miles broad rather than a line. It is not determined alone by altitude, by topography, by character of the bedrock, or by climate; it is the resultant of all of these. For the sake of clearness the foothills may be described as the drier and less elevated (about 5800–8000 ft.) part of the mountain plateau. with vegetation composed of grassland, scattered rock pines, and a few other trees (*foothill zone*, RAMALEY 8). Except in the southern "sedimentary plateau" (fig. 1), perhaps rather to be considered part of the mountain-front area, the foothills may be said to comprise the granitic hills of the mountain-mass proper; while to the mountain-front zone may be assigned the upturned sedimentary hogbacks and longitudinal valleys, sedimentary outcrops, buttes and broken plateaus, and the mesas and upper parts of the débris-covered slope to the plains. The vegetation is of the greatest variety. The plains proper may be said to commence where the mixed soil and

vegetation of the detrital outwash from the hills is succeeded by the fine soil and mostly short-grass vegetation of the shale beds covering most of the Great Plains surface.

Plan of presentation.—The writer has been much influenced by the work of DAVIS (1) on the geography of the Colorado Front Range, a regional presentation and particularly relevant in this study, since the area considered is so nearly the same. DAVIS' systematic treatment avoids repeating descriptions of frequently encountered land-forms by recognizing their common features and giving each a brief characterization and a name, thus identifying them when mentioned later. Minor differences of detail are not considered in the condensed treatment thereby made possible. In a regional study, in which numerous elements form an intricate complex, this omission of detail is essential. As the physical geographer refers land-forms to types (mental counterparts of physical realities), so in a regional study of plant geography one may refer forms of vegetation to types which are the same over considerable areas. This is a common practice in ecological classification, but many studies of limited areas of vegetation have characterized the plant communities without regard to geographic orientation. If possible, local representations or variants of widespread associations should be recognized as such. The characterization of the relatively few widespread and important vegetation-types makes it possible to systematize plant geography. This systematic treatment emphasizes the common features, the resemblances of similar plant communities, but the differences, when worthy of note, can always be stated in addition. The section of this study which is here published is the systematic part, which establishes the types of topography, soil, climate, and vegetation as developed in the region or in parts of it. It will be followed by a regional section, which describes the physical and vegetational features "in their actual spatial relations," to use the words of DAVIS, and by parts dealing with general geographic and developmental relations of the vegetation.

Physical features

The area studied is the eastern front of the Rocky Mountains in Colorado, of which the most characteristic part is the Front

Range. This has been studied by many geographers, more recently by DAVIS. The Front Range has been described by him as a submaturely dissected upland of crystalline rocks, elevated above the plains to the east by a long north-south monoclinal fold. The tops of most of the hills form the remains of a peneplaned surface, the result of the erosion following the uplift, with complete removal of the sedimentary layers from the raised area on the west. A few

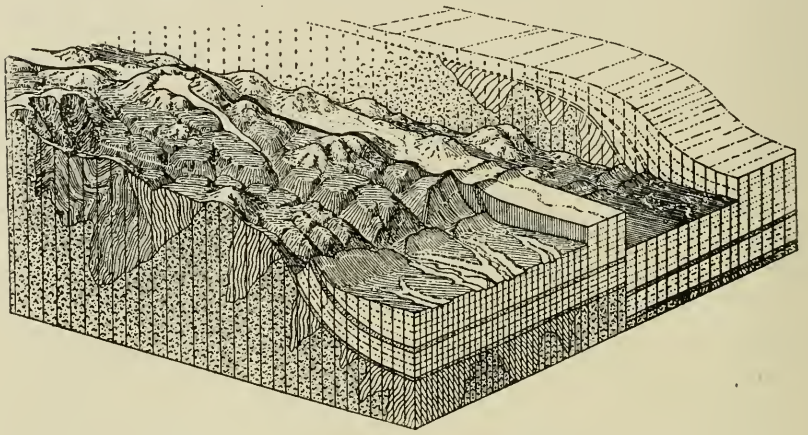


FIG. 2.—DAVIS' block diagram of Front Range (reproduced with author's permission from 1): at right is condition following first uplift with monoclinal fold; next part shows peneplaned upland with monadnocks and cuestas (hogbacks); third shows entire region after second uplift; last block on left shows present condition, with glacier-carved range-crest, gently sloping, dissected, crystalline upland, of which lower and eastern part forms foothills, and mountain-front, with sloping crags, cuestas, and longitudinal valleys; outside may be seen débris-covered terraces and broad valleys of streams running out into plains.

monadnocks surmount the general level. The present eastward inclination of the old peneplain and its dissected character in the crystalline area, and the removal of sedimentary strata of the plains to a depth far below the foothills, are the effects of a second uplift, an uparching of the whole region, and of the subsequent cycle of erosion. Near the base of the original fold the sedimentary strata are sharply upturned against the outer granitic slopes, the ends of the resistant strata forming ridges and sloping crags (fig. 2).

The outer slope to the plains has been described by JOHNSON (5) as a débris-apron or composite of alluvial fans, of which the profile is that of a stream-grade, rapidly flattening into the very slight and uniform incline of the Great Plains. The graded surface is covered by unassorted rock-waste from the hills, which thins out and becomes finer in texture toward the east; it is absent from most of the surface of the plains, which is of fine grained residual soil. This grade is that of the High Plains;[1] the streams have very generally cut below it, especially near the mountains. The Platte and Arkansas rivers, the trunk-streams, have cut very broad valleys in the soft shales of the plains. The north-south valleys of their tributaries which parallel the mountain-front are bordered on the east by escarpments of considerable height and are notable geographic features. This recent downcutting, where working in soft shales just outside the foothills, leaves many terraces, remnants of the older and higher stream-grade levels; their covering of rock-waste preserves their flat tops. They are generally known as "mesas"; although not true mesas, the term is convenient.[2] Where the upper sedimentary beds consist of sandstone or limestone, extensive plateau areas with deep canyons, buttes which may be numerous or scattered, or simple escarpments may be encountered. In a few places igneous intrusions are seen as dikes or as basaltic layers capping large mesas (true mesas in this case). From these features the mountain-front zone derives its varied character; the mountain upland on the west, and the plains extending far to the east, are of less irregular structure.

Arrangement of the component ranges and smaller ridges *en echelon* is a notable feature of the easternmost line of mountains. Ranges which are in general north and south of each other are themselves oriented with the northern end a little to the west. MARVINE writes (7, p. 132):

[1] The distribution of the remnants of the High Plains may be seen in a map by JOHNSON in the article mentioned.

[2] A true mesa is a tableland capped by a more resistant stratum which keeps the top flat by retarding erosion except on the sides. The débris-covered terraces flanking the mountains are like a true mesa in that the rock-waste layer acts as a more resistant cap.

In traveling from the north along the zone of hogbacks lying at the base of the mountains southward, the traveler finds the mountain-slope directly west of him falling lower and lower until it becomes an insignificant ridge, and finally dies away in the plains.

Passing around the southern end of the diminishing ridge the main mountain-slope is found lying several miles to the west, and separated from the ridge by a baylike valley extending northward behind it. . . . . The ridges are uplifted or anticlinal folds, the valleys depressed or synclinal folds, both dying away southward into the flatness of the plains.

The minor embayments due to echelon arrangement may be made out only in a large scale map, but the major embayments at the south end of the Rampart Range, the Pike's Peak highland, and the Greenhorn Mountains can easily be seen in fig. 1.

A more detailed view of the typical land-forms and vegetation-forms encountered in passing from mountains to plains traverses the several north-south zones in the following order: first the granitic foothills; then the transition zone of the mountain-front, with its upturned ridges, its mesas and graded slopes, and in places its plateau areas, buttes, and escarpments; and lastly the plains themselves.

GRANITIC FOOTHILLS

The mountain plateau is in most places submaturely dissected, the original upland level being represented only by the rounded tops of the hills (fig. 3). Slopes and summits are thinly covered with rock-waste. Occasional resistant dikes and ledges give craggy exposures of massive rock, not covered by any soil or débris. Below these, or on the sides of steeper ravines, are talus slopes of variously sized rocks, or slides of "granite-gravel."[3] Table I is a synopsis of topographic areas of the foothills arranged as habitats, and, correlated with these, the characteristic vegetation-types. Edaphic conditions largely determined by topography (local position in relation to surroundings, direction, amount of slope, and soil texture) have been discussed in the account of foothills vegetation (18).

This two-column form of presentation is adopted as being concise, as emphasizing relations between physiographic and ontographic features (the environment and the environed), and as permitting a more comprehensive view of the whole complex and its

[3] Decomposed granite in small angular fragments.

parts than can be obtained by the linear arrangement. Geographers will note that topographic *areas* rather than *land-forms* are used as the units of area of physical conditions (habitats), since land-forms, such as mesas and ravines, may include several topographic areas presenting quite diverse environmental conditions. Moreover, a single topographic area, even if physically uniform, may allow the growth within it of several more or less distinct vegetation-types.

FIG. 3.—Maturely dissected foothills near Boulder Creek: pine-sprinkled, rather than forested, surface mostly covered with dry grassland.

A brief statement concerning mountain parks may be made. These are small plains or flat valleys shut in on all sides by hills. They are not well developed in the foothills as compared with the montane zone. They are mostly formed where one of the principal eastward flowing streams is joined by tributaries from valleys opening into the park. There is a single outlet. Many of the montane parks in the Front Range contain the terminal moraines of former valley glaciers from above, and their topography is in large measure the work of ice. The slight gradient causes many

TABLE I

TOPOGRAPHIC AREAS (HABITATS) AND ASSOCIATED VEGETATION-TYPES IN GRANITIC FOOTHILLS COMPLEX

TOPOGRAPHIC AREAS	VEGETATION-TYPES
The geographic mean is that presented by rather exposed and xerophytic sloping surfaces, thinly covered with rock-waste of mixed texture, rather gravelly and with surface rocks. Local departures from the general condition are as follows:	The general ground-cover is mixed foothills grassland and primitive grassland, largely of grasses and herbs of the plains, with admixture of Rocky Mountain herbs, not all xerophytic. Scattered rock pines and plants of the mixed shrub association, singly or in clumps, dot the surface. In special habitats occur:
1. Exposed rock surfaces (bowlders and rock-walls)	1. Xerophytic lichen association
2. Rock-crevices	2. *Selaginella*, shrubs of *Jamesia* and *Ribes*, rock pine
3. Rock-strewn detritus slopes	3. Mixed grassland, and mixed consocies of primitive grassland, with higher proportion of woody plants (rock pines, mixed shrub, *Ceanothus*, *Arctostaphylos*)
4. Rock-talus	4. *Artemisia frigida-Koeleria* consocies of primitive grassland (18)
5. Compacted granite-gravel floors and side-slopes	5. Compacted granite-gravel consocies of primitive grassland (18) with rosette plants; *Arctostaphylos*
6. Loose granite-gravel floors, washes, and talus (gravel-slides)	6. Primitive grassland, with *Geranium-Chrysopsis* consocies, mat (rosette) consocies of gravel-slides, etc.
7. Mixed-soil floors and detritus-slopes (fine soil with imbedded and superficial rock-fragments of various sizes)	7. Foothills mixed grassland, with addition of other components, *Ceanothus*, sumac, pine, etc.
8. Fine-soil floors and detritus-slopes (infrequent)	8. Foothills mixed grassland, of a form approaching plains short-grass
9. Less xerophytic side-slopes (mostly north-facing, mostly of considerable gradient, and best developed in valleys)	9. Mixture of mixed shrub, rather less xerophytic mixed grassland, and pine associations, with representatives of canyon forest and scattered trees of *Pseudotsuga*
10. Narrow mesophytic ravines (best developed as small side-canyons, especially on the south side of eastward flowing main streams)	10. Mesophytic representations of mixed shrub, *Pseudotsuga*, aspen, *Symphoricarpos*, canyon forest, and mesophytic grassland associations. Mosses, *Saxifraga*, etc., in wet rock-crevices
11. Stream-sides in shaded ravines	11. *Betula*, *Alnus*, *Corylus*, and *Acer glabrum* of the canyon forest; shrubs; moist-soil herbs, as *Heracleum*, *Rumex*, etc.
12. Stream-sides in open canyon bottoms	12. *Populus angustifolia*, willows, etc.

meanders and oxbows in the streams, and there are in some parks small lakes in morainal depressions. The stream-sides are frequently boggy, with meadows adjoining. The parks are mostly treeless, or nearly so, and show no signs of former or impending forestation. The exposed dry flats are covered with dry grassland, its composition depending on altitude and geographic position chiefly. Differences in soil texture cause local variation of the grassland, but this is less marked and less minutely local than on the hill slopes. Certain lower areas are occupied by meadow and sedge communities, and the rolling surfaces of moraines (in montane parks) are variable in soil texture, soil moisture, and in the composition of their grassland cover; but the greater part of park floors is well drained, flat, and quite uniformly covered with dry grassland. This vegetation, in any one park, forms what might be called a crystallization of the grassland of the neighboring hills, whether in foothills or montane zone, in view of the comparative uniformity of the grassland of the flats as contrasted with that of the diversified slopes of hill topography. The lower parks have a grassland cover very like that of coarse soil in the mountain-front area or in the plains (see description of Estes Park in the regional section). The higher parks have fewer plants of the plains and more of the mountains. There is a floristic and vegetational gradation from plains grassland through the lower parks to montane grassland as seen in the higher levels. The parks thus show a steplike series of floristic and ecological changes with altitude. RAMALEY (10, 11) for some years has studied park vegetation, especially in Boulder Park at Tolland, Colorado, on South Boulder Creek.

TRANSITION AREA OR MOUNTAIN-FRONT ZONE

The sedimentary rocks, lying upon the granite, are upturned at the monoclinal fold, and are seen in a horizontal series of exposures of strata, the lower and older members abutting on the granitic foothills to the west, the upper formations outcropping in order toward the east. Since the tilting at the mountain-front is for considerable distances greater than 45° (locally reaching 90° and even more, resulting in overturns), the lower formations have narrower zones of outcrop than the upper strata, which dip so slightly as to cover areas many miles wide in the plains. The

narrow zone of older and lower strata contains alternating resistant and soft members, giving rise to the hogback ridges and intervening valleys already mentioned, while the newer rocks are mostly soft shales and sandstones, giving a flat or rolling topography over the surface of the plains, with occasional escarpments at the edges of stream-valleys. Both angle of dip and hardness of rock, therefore, contribute to a differentiation, in the sedimentary area outside the foothills, of a relatively narrow ridge-valley mountain-front zone from the very broad and mostly flat plains region.

Just outside the ridge-and-valley zone is the graded slope to the plains, covered with rock-débris and dissected into terraces or mesas of varying level. In places along the mountain-front the ridge-and-valley topography is absent or poorly developed, either because the troughs are not yet carved beneath the slope from the granitic hills, or because the ridges are already planed (locally) to a graded floor. The terraces are also missing from certain parts of the mountain-front. The topographic complexes of the ridge country and of the mesa country may now be described separately.

The hogback ridges (cuestas) and intervening troughs (figs. 4, 5).—Two of the numerous sedimentary strata overlying the crystalline rocks are so resistant as to form ridges over great lengths of the mountain-front. These two strata are of such conspicuous geographic importance that they merit distinctive names and since many persons know them by their geological names, these will be used here in a geographic capacity. The *Fountain* sandstone, which in most places lies directly upon the granites, is very thick, and is composed of dark red, rough arkose materials, variable in texture. It is in places more resistant than the granites, so that side-gulches tributary to the east-flowing streams of the foothills are common in the granite just beneath the Fountain. Continuous troughs between the Fountain and the granite are not frequent. In many places the hard red sandstones form broad smooth-faced crags lying upon the outer foothill slope, reaching maximum size in the well known "flat-irons" south of Boulder (fig. 6). The other hard stratum is the massive gray sandstone known as the *Dakota*. It is separated from the Fountain by several less resistant strata of considerable aggregate thickness,

FIGS. 4, 5.—Upturned sedimentary ridges of mountain-front zone: fig. 4, eastward view in Perry Park, where a broad flat valley has been leveled between ridges and outer granitic foothills; floor of flat is of compacted angular fragments; vegetation is primitive grassland alternating with scrub oak; Dawson Butte in far background; fig. 5, southward view, between Golden and Morrison, of longitudinal valley inside Dakota hogback, shown on left in long curve.

and is usually seen as a bold ridge parallel to the outer slope of the foothills some distance to the east. The term "hogback" is familiarly applied to the steep Dakota cuesta.

A deep and wide trough usually extends between the Fountain crags and the Dakota cuesta. The upper part of the east-facing slope of this trough is the outcrop of a "creamy sandstone," which in places forms prominent outcrops, or even strong ridges, as at Morrison at the mouth of Bear Creek. Just east of and below the creamy sandstone is an easily eroded shale, which gives its rich red color to the deep soil of the valley. The west-facing slope, below the Dakota crest, is the outcrop of a calcareous sandstone stratum which is weathered so slowly as to be covered only by a thin soil. In certain places this limy sandstone stratum is hard enough to form a separate ridge or hogback crest.

The Dakota hogback is one of the most constant and conspicuous topographic features of the mountain-front, since it is practically everywhere harder than the strata above and below. Its top is usually quite even and straight, representing the level of a former graded surface. Its crest is quite rocky; there is no soil except in the crevices.

The present graded slope to the plains begins usually with the outer slope of the Dakota hogback, through first a layer of dark shales, then a thin limestone overlaid by soft light-colored shales, then clays and shales. Near every east-flowing stream, however, the graded slope is likely to be cut beneath by side-gulches cutting down into the dark shales, leaving a cut-off mesa with the limestone at its high western end.

Local distribution of vegetation in the mountain-front belt of upturned sedimentary rocks presents a variability apparently dependent almost entirely upon topography and soil texture, just as in the area of granitic foothills. There seem to be few if any perceptible differences in the floras of the different geological formations which can be traced to chemical differences in the substratum. It is perhaps true that cedars are more frequent in the limestone or calcareous sands of the stratum just below the Dakota, where these are exposed in gulches which notch the Dakota hogbacks, and that there are certain slight floristic differences between

granitic and sedimentary areas. This question has been discussed by RAMALEY (9), who found the two areas about the same in floras (in the Poudre mountain-front area), with *Cercocarpus* abundantly represented in the sandstone but not in granite, *Selaginella* apparently absent from the sandstone, and lichens infrequent there. Following a suggestion from COWLES, it appears to the writer that differences in rate of erosion of the substratum may explain the distribution of lichens, and perhaps *Selaginella* also. The sandstones are rather soft in the Poudre area, and wear away too rapidly for the lichens to establish themselves abundantly. The Fountain sandstone is harder in the Boulder region than elsewhere, and there at least it bears lichens almost as abundantly as do the granites. *Selaginella* is frequent in the sedimentary rocks in the Boulder area, as RAMALEY has pointed out. The writer knows of no plants which are restricted to either sedimentary or granitic areas, the only observed differences being those of relative abundance. The gulches, exposed slopes and crests, etc., of the sedimentary area are quite comparable to similar topographic situations of the granitic foothills, and have practically the same plant assemblages.

The rocky upper slopes of the Fountain, the Dakota, and other ridge-making strata, where they occur, lack soil except in crevices, and are mostly bare, except where rock pines or pinyons, shrubs of rocky situations (*Cercocarpus*, *Ribes*, *Jamesia*, etc.), and crevice plants, including many xerophytic herbs, can obtain a foothold. The west slope of hogbacks is blufflike, usually, and rocky, while the east slope is less steep (depending on the local angle of dip) and likely to be strewn with débris, as are the slopes of the harder exposures of the valley, and these have shrubby or herbaceous vegetation, sparse, and of species of rocky situations. The softer shales occupying the bottom of the valley are usually deeply buried by débris (of fine soil with imbedded rock fragments of all sizes), and support a grassland vegetation, which is luxuriant in the rainier parts of the growing season and very dry the rest of the time. A stream-bed in the bottom of the valley may be bordered by a strip of mixed shrub, *Crataegus*, oak, or canyon forest; or if dry, by scattered narrow-leaf cottonwoods and willows. Mesophytic

ravines developing in the sedimentary area support mixed-shrub, woodland, or mesophytic herbaceous growths, as in the granitic foothills. Local meadows (mesophytic grassland) are found on slopes where seepage or a high water table moistens a deep soil for at least part of the growing season.

In places the sedimentary rocks have been worn down more than is common, so that they are mostly or in part reduced to a general grade, above which the more resistant layers rise locally. This is the condition in the valleys of some of the larger streams from the foothills, and is seen at Platte Canyon, partially at Bear Creek (Morrison), and also in Perry Park (fig. 4) and the Garden of the Gods. The floor of this graded surface, especially in the Fountain exposures, is likely to be covered very thinly with small angular fragments, loose or compacted. The vegetation, as well as the soil, is very like that of gravelly floors in the foothills, being a variant of the primitive grassland association, with scattered rosette or mat plants, *Bouteloua hirsuta*, etc.

The climatic transition in the zone of upturned sedimentary strata is rapid. At Boulder and elsewhere dense cloud-banks have frequently been seen to descend to or just beneath the Fountain crags without continuing outward and downward to the plains (figs. 6, 7). The outer granitic hills and upper sedimentary slopes receive greater and more frequent precipitation than the lower slopes and adjacent mesas and plains; it may rain slightly below while it snows considerably above (cf. fig. 12); the outer and lower slopes are more exposed to wind, less cloudy, and in places less shaded from the afternoon sun by the higher granitic hills than the inner valleys and upper slopes. No exact data are available for this sudden climatic transition. Where the outcrop of sedimentary ridges and valleys is wide, as in the northern mountain-front region, the outer hogbacks are severely exposed to sun and wind, as in the open plains. Their coarse rocky soil favors woody plants; the xerophytic *Cercocarpus* shrub assemblage is here more extensively developed than anywhere else.

MESAS AND GRADED SLOPES OF THE DÉBRIS-APRON (fig. 8).—The general character of the graded slopes and their mesa-fragments has already been suggested. The mesas are of varying ages and

levels. They are described in the accounts of Lee (6), Johnson (5), Fenneman (2), Shantz (15), Ramaley, Robbins, and Dodds (12), and Vestal (17). The topographic parts of a mesa are: (1) the

Figs. 6, 7.—Climatic transition at mountain-front: fig. 6, outer mountains just south of Boulder, seen from university campus; clouds beginning to form at summit of Green Mountain, while much of South Boulder Peak, at extreme left, is already obscured; snow covers the mountain slopes and fades out toward base of high mesas; roofs of distant buildings also white; fig. 7, practically same view, a little later, with upper slopes obscured; at one time it began to snow on mountains and upper mesas, and a few minutes later to rain in town; shortly afterward it changed to snow in the upper edge of town, so that the roof of the building with the short steeple at the right in midground, and of nearer houses, were well whitened, while rain still fell on the campus, less than half a mile away, and not more than 50 ft. lower; difference in elevation at mountain-front is critical as regards climatic change.

mesa-top, with flat surface covered with mixed rock-débris; (2) the edge or mesa-crest; (3) the side-slope; and (4) valleys or draws in the side-slope. The soil conditions and their effects on plant distribution have been discussed in the three articles last cited.

The débris-cover, where it has not been removed by recent erosion, extends far out into the plains. Its removal from the extensive areas of soft shales and clays marks a change from the flat terrace level to the easily eroded, gently rolling surface of much of the plains. The High Plains are extensive remnants of the old graded surface, away from the mountains.

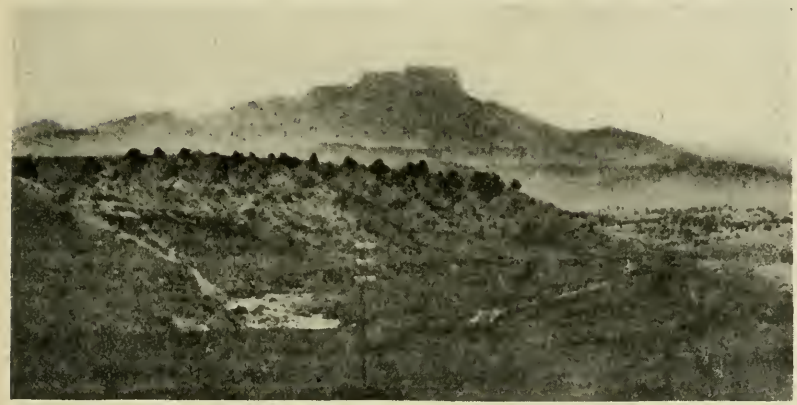
The north-south distribution of the terraces is practically that of the mountain-front, although as conspicuous topographic forms the mesas are not so extensive. So far as effects on distribution of vegetation are concerned, the presence of the coarse mixed soil of

FIG. 8.—Table Mesa, about 7 miles north of Boulder; outlines of hills sketched in with ink; ridge DDD is Dakota hogback; Boulder mesas may be seen in figs. 6 and 7.

the detrital surfaces is the important physical condition. It permits the growth in the same small area of a rich variety of plants, representing numerous vegetation-types and different geographic elements.

PLATEAU AREAS, BUTTES, AND ESCARPMENTS (figs. 9–11).—Where the sedimentary strata are horizontal or of rather slight dip the harder layers protect the softer rocks beneath, and extensive plateau surfaces are left above the grade established by present erosion. These can be invaded only at the edge and by ravines which eat their way headward into the bluffs. Smaller elevated areas or buttes, recently or long ago cut away from plateaus by

meeting of two such ravines, are common. Older buttes are fewer and more distant from one another.



FIGS. 9, 10.—Buttes and plateau areas: fig. 9, North Table Mountain at Golden, west of Denver; this and South Table Mountain are capped with basalt; fig. 10, Fisher Peak, northern end of Raton mesas, as seen from valley of Purgatoire River, a few miles above Trinidad; upland in midground belongs to southern sedimentary plateau; vegetation is principally dry grassland with scattered pinyons and cedars and infrequent clumps of scrub oak.

The plateaus and buttes are found outside of the upturned ridge and valley zone, wherever the surface rocks are rather resistant. These resistant strata are usually the most recent and uppermost,

although the much older Dakota is at the surface over considerable areas in the plains drained by the southern tributaries of the Arkansas. For a considerable thickness above the Dakota the strata are mostly soft shales, which erode too readily to give tableland topography.

The larger through streams and their tributaries have cut below the level of the High Plains, leaving escarpments which are particularly notable near the Platte-Arkansas divide. Plum and

FIG. 11.—Buttes and plateau areas: divide between East Plum and West Plum creeks, in Castle Rock area, showing some of rhyolite buttes; one of the most imposing of these, Dawson Butte, shown in fig. 4.

Cherry creeks, running north into the Platte from the divide, and Monument, running into Fountain Creek, south to the Arkansas, have eroded deep valleys parallel to the mountain-front. Away from the mountain-front proper these valleys are bounded by lines of steep cliffs, but the west border of Monument and West Plum Creek valleys is the graded slope from the foothills, with its débris-covered terraces. Isolated buttes are present within these valleys, some of them protected by caps of igneous rocks from local outflows.

The southern part of the Sangre de Cristo Range (sometimes considered as a separate mountain chain, the Culebra Range) is flanked on the east by a sedimentary plateau which rises abruptly above the plains in a steep line of bluffs. The plateau is of sandstones mostly, of slight dip, and is much dissected by the eastward

flowing streams and their tributaries. On it rests the highland of the Spanish Peaks, and it is ribbed by resistant dikes of igneous material from two outflows, one set radiating downward from the peaks themselves. With the plateaus should be classed the high lava-capped mesas of the mountain-front and plains in the area near the Colorado–New Mexico boundary.

As in the hogback ridges, vegetation distribution in the plateau and butte areas is largely determined by soil texture and topography. Atmospheric conditions vary with exposure to wind and sun. The tops of the plateaus are covered with short-grass and

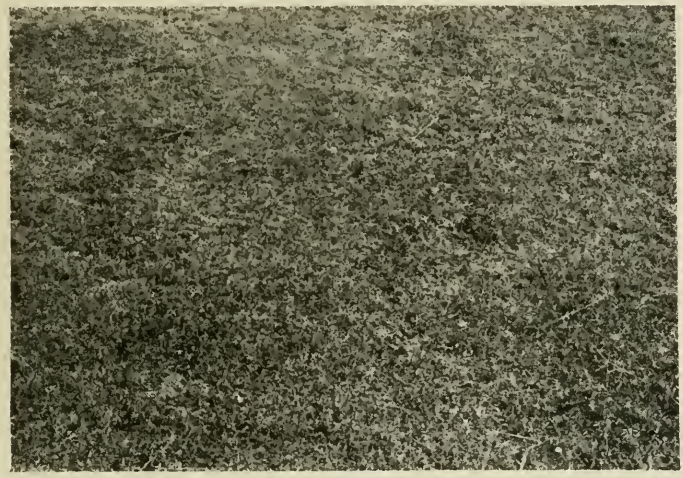


FIG. 12.—Unbroken short-grass ground cover in plains

mixed grassland over the level upland stretches of comparatively fine-textured soil. Exposed cliffs and crests, and rocky débris-slopes, afford lodging places for woody xerophytes (*Cercocarpus*, rock pines, pinyons, and cedars), with primitive grassland as the general ground-cover. The deeper and shaded parts of canyons and ravines approach a mesophytic condition, with mixed shrub and woodland vegetation.

PLAINS

Plains topography is typically flat or gently rolling country, with fine clay soil from a soft-shale substratum. Short-grass is the characteristic vegetation (fig. 12). Where the substratum is

sandstone the soil is more porous, with much sand; and plants of an assemblage typical of sandy soil are seen (17). Sand hills are present locally, usually to the leeward of larger streams.

Near the mountains the débris-cover, if present, considerably modifies topography, soil conditions, and vegetation. It may extend a long way into the plains, or may have been removed very near the beginning of the graded slope from the foothills.

Saline or alkaline areas are locally present. The valleys of the Arkansas and its tributaries (wet-weather streams, many of them, with trenched flood-channels) are in many places alkaline, and show prominent stands of *Sarcobatus-Chrysothamnus* vegetation.

Woody vegetation from the foothills extends locally far into the plains in rock outcrops, and along stony crests of stream-bluffs or terraces. The larger streams are bordered for many miles from the mountains by cottonwoods, usually scattered.

Climate

The region has a continental climate, semi-arid, less so at the base of the mountains and in the foothills, with most of the rainfall in the warmer months. Wind movement, proportion of sunshine, and evaporating power of the air are high in the plains, with wide extremes of temperature; all of these features are less marked in the foothills.

The southern part of the region is warmer and drier than the northern, and with different distribution of rainfall. The rapid east-west change in elevation and topography at and near the mountain-front is accompanied by more or less considerable climatic variation; this with the local peculiarities occasioned by the elevated Platte-Arkansas divide, and the differences between areas north and south of the divide, may be seen in the summaries of climatic data for the particular subregions. These data have been taken from the summary of Climatological Data for eastern Colorado, southeastern Wyoming, and northeastern Colorado.[4] The facts shown in table II should be considered in the light of their

[4] Section 6, northeastern New Mexico, by C. E. Linney. Section 7, region drained by the Arkansas in Colorado, and section 8, region drained by the Platte in Colorado, by F. H. Brandenburg. Section 24, southeastern Wyoming, by W. S. Palmer.

determinative influence upon the vegetation; this can be done in only the barest manner in this section, but these relations are again brought out in the part on geographic relations of the vegetation.

Temperature conditions of the different parts of the region may be summarized as follows: The foothills have a lower mean temperature and shorter period without frost than either plains or mountain-front. Certain of the foothills vegetation-types and

TABLE II

TEMPERATURE DATA

Area	Average mean temperature ° F.	Maximum temperature	Minimum temperature	Average number of days in growing season
Foothills (4)	43.1	100	−36	99
Northern (2)	42.0	98	−32	95
Southern (2)	44.3	100	−36	104
Mountain-front (5, excl. Divide)	50.5	104	−30	154
Northern (1, Boulder)	50.9	97	−20	164
Divide (2)	46.7	99	−33	122
Southern (4)	50.3	104	−30	151
Plains near mountains (5)	47.7	105	−38	138
Northern (3)	47.1	105	−38	134
Southern (2)	48.6	103	−32	143
Dry plains (5)	50.4	106	−45	151
Northern (2)	48.6	103	−45	145
Southern (3)	51.0	106	−32	156
"Northern area" (8)	45.8	105	−45	131
"Southern area" (11)	49.3	106	−36	142

The number of stations for each area is given in parentheses. The mountain-front does not include the two stations of the Platte-Arkansas divide, which is so much more elevated than other parts of the mountain-front as to be much cooler. The "northern and southern areas" are respectively the northern and southern parts of the region, each extending over foothills, mountain-front, and plains.

many of the plant species are characteristic of northeastern and northwestern coniferous forest regions, are in fact southern extensions of them. The boreal character is much more evident in the higher mountains than in the foothills.

The mountain-front has the longest frostless season, the highest mean temperature, the mildest winters, and the least range in temperature extremes. Mountain-front localities are mostly comparatively sheltered; temperature inversion is common. Early spring plants flower several weeks earlier at the mountain-front than in either plains or foothills; at Boulder in spring the

season is in general 2–3 weeks in advance of that of Denver, 14 miles from the mountains.

The divide between Platte and Arkansas drainage, which should be considered in connection with the mountain-front area, has a mean temperature and frostless period intermediate between those of mountain-front and foothills areas, as it is intermediate in altitude and in vegetation.

The plains have a slightly lower mean temperature and shorter season without frost than the mountain-front area; the temperature of the dry plains at some distance from the mountains approaches that of the mountain-front more closely than that of the plains adjoining it. This difference is accompanied by a floristic one. Temperature extremes are greatest in the plains, a condition inimical to growth of woody plants.

The plains, mountain-front, and foothills in northern Colorado ("northern area") are cooler than those to the south, but the north-south differences in temperature and length of growing season due to latitude are of much smaller range and influence upon vegetation than the east-west differences due to altitude and changes of topographic character.

For purposes of comparison table III includes rainfall data for the higher parts of the mountains bordering the foothills on the west (montane zone), and for the plains of eastern Colorado bordering the region studied on the east. Annual rainfall is higher to the west, increasing with elevation, and higher also in the eastern plains, as a part of the gradual geographic increase of rainfall from the dry belt of the Great Plains eastward through the prairie region to the border of the eastern deciduous forest region. The eastern plains mark the transition from short-grass plains to the taller prairie-grass vegetation of the prairie, and are known in Colorado as "the rain belt." The driest part of the plains region lies between the rain belt and the plains near the mountains, in a zone distant from the mountains about 18–25 miles, and of a breadth 30–60 miles. It is narrowed on the west by the elevation of the Platte-Arkansas divide, and extends farther eastward in the Arkansas River Valley. It extends only a little way north into Wyoming and apparently is much narrowed on the west in extreme southern

TABLE III

Rainfall data

Area	Annual	January	February	March	April	May	June	July	August	September	October	November	December
Montane zone (6) 8250–10,265	**22.58**	0.80	1.10	1.96	2.75	2.85	2.01	3.38	2.70	1.61	1.64	0.80	0.99
Foothills (10) 6890–8000	**16.30**	0.42	0.60	1.11	2.17	2.23	1.27	2.79	2.06	1.29	1.27	0.55	0.44
Northern (6)	16.75	0.42	0.68	1.38	2.26	2.88	1.47	2.20	1.90	1.29	1.31	0.47	0.49
Southern (4)	15.63	0.44	0.49	0.69	2.03	1.25	1.18	3.67	2.29	1.27	1.23	0.68	0.37
Mountain-front (13) 5069–7373	**16.25**	0.40	0.68	1.07	2.06	2.59	1.79	2.41	1.81	1.23	1.11	0.53	0.54
Northern (5)	16.95	0.46	0.68	1.48	2.64	3.11	1.56	1.99	1.29	1.31	1.29	0.55	0.52
Divide (3)	16.53	0.35	0.56	0.94	2.08	2.69	2.17	2.73	1.96	0.77	1.11	0.47	0.69
Southern (5)	15.38	0.35	0.74	0.72	1.47	2.01	1.78	2.69	2.25	1.37	0.93	0.56	0.48
Plains near mountains (6) 4985–6098	**14.36**	0.33	0.49	0.81	1.85	2.28	1.76	2.17	1.81	1.20	0.81	0.47	0.43
Northern (3)	14.22	0.45	0.51	0.97	2.02	2.70	1.57	1.87	1.34	1.05	0.90	0.43	0.44
Southern (3)	14.54	0.20	0.47	0.65	1.67	1.86	1.94	2.47	2.27	1.34	0.72	0.51	0.43
"Northern area" (14)	**16.28**	0.44	0.64	1.33	2.20	2.92	1.53	2.06	1.57	1.27	1.22	0.50	0.49
"Southern area" (12)	**15.25**	0.35	0.59	0.69	1.46	1.72	1.56	2.96	2.27	1.33	0.93	0.59	0.43
Dry plains belt (7) 3899–5400	**12.73**	0.26	0.39	0.74	1.62	2.17	1.49	2.26	1.67	0.79	0.75	0.29	0.36
Eastern plains (8) 3380–5178	**16.95**	0.35	0.53	0.76	2.01	2.59	2.58	2.89	2.06	1.29	0.87	0.52	0.52

The northern area is the aggregate of northern foothills, mountain-front, and plains; the southern area is its southern equivalent. The number of stations is given in parentheses for each area, and the range of altitude above sea-level of the several north-south zones (in feet) is also included. The rainfall figures are in inches.

Colorado and northeastern New Mexico by the lava-capped plateaus which there extend eastward from the mountains.

As for the mountain-front and adjacent foothills and plains, the first two average about the same, the mountain-front receiving slightly more rainfall in the north and at the divide than the foothills. This may perhaps be due to the fact that in the northern part of the state, as at Boulder, the rain usually comes with east and northeast winds; and since the change of elevation is greatest at the mountain-front, more precipitation might occur there than in the foothills beyond. At Boulder the more mesophytic forms of vegetation occur more frequently and in larger areas in the sedimentary rocks of the mountain-front than in the granite foothills half a mile or a mile inside the foothills. In the southern part of the state the mountain-front is drier than the foothills, as a rule. The plains near the mountains receive almost 2 inches less rain, on the average, than foothills and mountain-front, and the dry plains to the east nearly another 2 inches less.

The "northern area" (foothills, mountain-front, and adjacent plains) receives on the average about 1 inch greater rainfall than the "southern area." Coupled with the higher temperature and greater evaporation, this results in a considerably more xerophytic vegetation south of the Platte-Arkansas divide. There is little difference in the plains, but at the mountain-front, with a difference of 1.57 inches, the vegetation to the south is markedly drier.

COOPER finds, in the chaparral region of California, that very slight differences in the original physical conditions of north and south slopes result in very marked differences in vegetation. The same principle seems to apply, in perhaps a smaller degree, in a semi-arid region like the Colorado mountain-front. It appears that differences in rainfall from place to place, or from month to month, although small in absolute amount, can be critical in their influence upon vegetation distribution. The slight differences appear to represent marginal values above or below a critical point. The difference in vegetation in two areas, moreover, is not necessarily the result of climatic difference, but is a resultant of differences in soil, topography, geographic position, and vegetational history, in addition to climate. It should not be surprising, therefore, that

areas having climates not widely dissimilar, as the plains of the rain belt and the northern foothills, should have distinctly unlike vegetation.

Minimum rainfall.—One factor which seems to be partly responsible for the generally xerophytic character of the entire region studied, the plains in particular, is the wide variation in the amount of rainfall from year to year. The minima have been

TABLE IV

Minimum annual rainfall

Area	Number of stations with records		Average minimum for area		Lowest minimum recorded for any station in area	
	1893	Other years	1893	Other years	1893	Other years
Montane zone	—	6	—	**15.65**	16.55 Frances	**11.36** (1907) Cripple Creek
Foothills	4	9	—	**12.83**	**7.16** Box Elder	10.93 (1908) Cheesman
Mountain-front and divide	7	11	9.12	**11.91**	**7.03** Waterdale	8.76 (1890) Table Rock
Plains near mountains	5	6	9.39	**8.91**	7.11 Fort Collins	**5.04** (1876) Cheyenne
Dry plains belt	7	7	8.11	**7.01**	5.40 Greeley	**3.78** (1894) Las Animas
Eastern plains	5	8	10.48	**10.61**	8.30 Cheyenne Wells	**6.97** (1894) Cope

Two stations within the foothills area are exceptional as to rainfall, and have not been included in the averages. These are Salida in the Arkansas Valley above the Royal Gorge, and Westcliffe in the Wet Mountain Valley. Similarly, Canyon City at the debouchure of the Arkansas, and Raton and Las Vegas in New Mexico, have been excluded from the mountain-front area. The stations with the lowest minima have been mentioned in the table. The lowest minimum in each area, whether in 1893 or in some other year, is printed in bold face. Except for Cheyenne Wells, which is remote from the mountains, all of the stations noted as having had least rainfall in 1893 are within a limited area (in the northern part of the region), which seems to have been most severely affected by the drought of that year.

tabulated for the several parts of the region from the climatic summaries of the Weather Bureau. The year 1893 happened to be exceptionally dry, and the minima for many of the stations fall in it. Dryness in other years has been of more local prevalence. It has seemed preferable to present the data for 1893 separately from that of other years. The data for 1893 are not available for all stations in each area, and so the number of stations from which data have been used is mentioned for each area (table IV). The column presenting the average minima for the several areas (minima

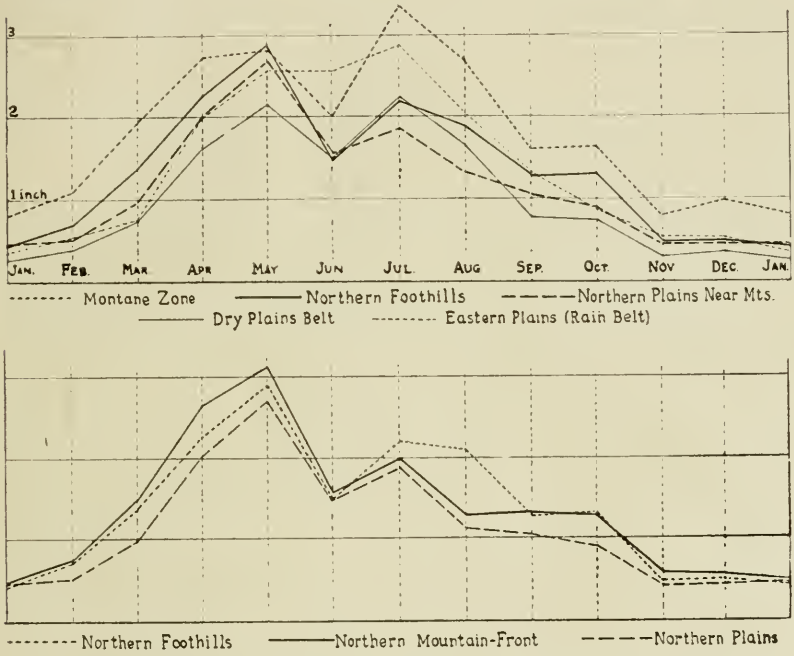
of all stations for each area averaged, excluding figures for 1893) seems to express the main fact of the table, that the rainfall reaches successively lower minima downward and eastward from the mountain zone through the foothills, mountain-front, and adjacent plains to the dry plains belt, beyond which the minima rise gradually with the gradual increase of rainfall eastward into the prairie-grass region. It appears also that annual rainfall values falling considerably below the average (as much as 4 inches below) occur more frequently in the plains than in the mountain-front and foothills areas. The well known uncertainty of farming without irrigation in much of eastern Colorado, due to frequency of very dry years, indicates further that it is not the average rainfall so much as the constantly recurring minimum which determines whether or not an area can support a cultivated or natural vegetation which is other than decidedly xerophytic.

SEASONAL DISTRIBUTION OF RAINFALL.—On the whole, precipitation during the cooler months is quite low; this is not so true of the montane area just to the west of and above the foothills. The summer rainfall is greater, but in most places distributed rather unevenly. June is thus drier than either May or July over practically the entire region. The northern area near the mountain-front receives more rain in the spring and early summer months, while the southern area receives more of its rain during late summer. This difference between north and south is of far-reaching influence upon the character and distribution of vegetation. The details of seasonal distribution of rainfall are shown in the table of averages of rainfall data, and in figs. 13–16.

The northern and southern parts of the zones at and near the mountain-front are so different as to rainfall that they cannot be incorporated in single graphs. The northern parts of the zones are selected, therefore, as the more typical. The graph for the mountain-front is omitted to avoid overcrowding, but it can be seen in fig. 14. Excluding the eastern plains, the zones have the same type of rainfall, with greatest abundance in May and July, and a decline in June. The zones are successively drier with decrease of elevation, and this is almost as true for particular months as it is for the entire year. The eastern plains have higher

summer rainfall than the plains near the mountains; the distribution is similar, except that June is as rainy as May.

The graphs for foothills and plains near the mountains are repeated in fig. 14. These zones and the mountain-front have maximum rainfall in May, with a sharp decline in June, followed by slightly greater rainfall in July. Despite its less elevated position, the mountain-front receives greater spring rainfall than the foothills.

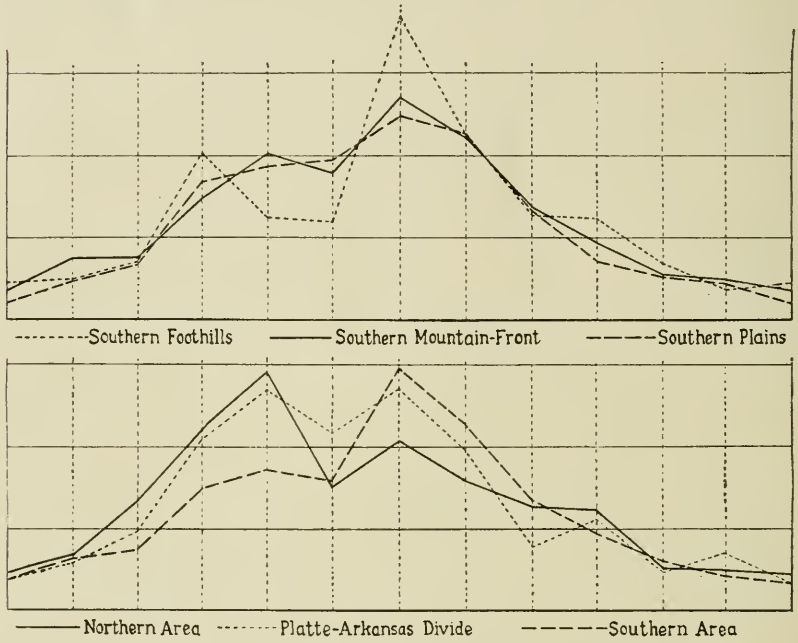


FIGS. 13, 14.—Seasonal distribution of rainfall: fig. 13, comparison of north-south zones; fig. 14, northern foothills, mountain-front, and plains.

The data for the south are not so dependable as for the north, for some of the few stations are exceptionally situated. The contrast shown with the northern area is marked, however. The rain is less abundant in spring and more abundant in July and August than to the north.

The graph shown for the "northern area" is a composite of the 3 in fig. 14, that of the "southern area" is a composite of those

in fig. 15; they contrast strongly. The northern area is characterized by the Rocky Mountain foothill type of rainfall, the southern area by the New Mexican type (WARD 19). Both of these types are described as having a single maximum, for the first in May, for the second in July-August. The northern area receives most of its rain from northeasterly winds; the southern area probably from southeasterly winds. The centrally situated Platte-Arkansas



FIGS. 15, 16.—Seasonal distribution of rainfall: fig. 15, southern foothills, mountain-front, and plains; fig. 16, northern and southern areas and divide.

divide receives rain from both directions, and has both maxima, with a higher June rainfall, partly because of its considerable elevation. Probably rain is carried from either direction past the divide, producing secondary maxima, in July in the northern area, and in April in the south.

The abundant rainfall of the divide, especially in June, forms a local rainfall type which is intermediate between that of well watered parts of the foothill zone and that of the eastern or rain-

belt plains. The divide is also cooler than most parts of the mountain-front and adjoining plains. The vegetation of the divide is likewise transitional between that of rain belt and foothills, with the more nearly mesophytic forms of grassland, and with woody plants of the foothills extending many miles eastward from the mountains.

The effects upon the vegetation of the difference in distribution of precipitation north and south of the divide are discussed in the section on geographic relations, but may be summarized briefly herewith.

TABLE V

INFLUENCE OF SEASONAL DISTRIBUTION OF RAINFALL ON VEGETATION

NORTHERN AREA	SOUTHERN AREA
The greatest rainfall is in April and May.	The greatest rainfall is in July and August.
There is greater activity of vegetation, more luxuriant growth, and greatest abundance of flowers in spring.	There is greater activity of vegetation and more luxuriant growth in late summer.
There are many spring-flowering plants from the mountains of rather mesophytic character, in mixture with plains plants in the mountain-front zone.	There is absence or scarcity of spring-flowering mountain plants, and greater prevalence of plains plants in the mountain-front zone.
Distribution of the bunch-grass association and of the less xerophytic plants, requiring a long season of continued moisture, is limited.	Distribution of bunch-grass is less restricted; there is a greater prevalence of late-flowering plants not intensely xerophytic, as some of the asters and goldenrods, etc.
The northern plains near the mountain-front flower luxuriantly in spring and early summer, but only the more xerophytic composites, etc., in late summer, in which respect the plains are more like the driest plains just east of them in late summer.	The southern plains near the mountains contain fewer spring flowers, but many long-season plants absent from the dry plains and the northern plains near the mountains are present, as the annual sunflowers. In this respect the plains are more like those of the rain belt of eastern Colorado in late summer.

It is remembered that the southern area is in general drier and warmer, with somewhat more xerophytic vegetation than the northern area, and that differences in vegetation due to this cause must be distinguished as well as possible from those due to different distribution of rainfall.

Evaporating power of the air has not been subject to geographic-statistical treatment, since there are no data. It was beyond the scope of the present study to have attempted instrumental investigation on a scale large enough to be of value. There seems to be little doubt that, as a geographic factor *in regions of continental climate*, evaporating power of the air is of about the same indicator value as rainfall. It varies geographically about as does rainfall, in inverse ratio, since evaporating power is, in large measure, a function of rainfall. This same inverse ratio seems to hold in seasonal distribution as well as geographically. This may be seen from the graphs of WEAVER (20), and from data obtained by COOPER in a study of chaparral in California.

As a local factor evaporation is separately treated in the discussion of local distribution of vegetation.

For further discussion of the climatology of Colorado in relation to vegetation the reader is referred to the articles of SHANTZ (15, 16), RAMALEY (12), and ROBBINS (13, 14). Data may be had from the bulletins of the United States Weather Bureau, Colorado College, the Agricultural Experiment Station at Fort Collins, and the Bureau of Plant Industry.

Local distribution of vegetation

PHYSICAL FACTORS.—Local physical conditions affecting plant distribution are those concerned with *substratum* and *soil;* with *topography*, especially *local position* with respect to surroundings, and *slope* of surface, as regards both steepness and direction of exposure; and with *local variation in atmospheric conditions*, as controlled primarily by topography. The variability of these factors within the region is great, and their interactions are complex. Descriptions of the soil, topography, atmospheric conditions, etc., of the different parts of the region are scattered through both systematic and regional sections of this study, and a lengthy discussion at this point would be out of place. A few references to other parts, and certain incidental comments, may here be made.

The character of the substratum, and some of its influences in determining soil conditions and topography, are indicated in the

account of the sedimentary area. The contrast between the granite soil of the foothills and the soil of sedimentary origin lying just outside, with its slight selective action on flora and vegetation, has also been noted. Other mentions of soils, especially as regards soil texture, are scattered.

Topography is systematically treated for particular regions and smaller areas by dividing each type of topographic complex into topographic areas or habitats; with each type is correlated the particular plant community or the several communities which accompany it. In the regional section will be found similar analyses of the cuesta, high mesa, mesa-terrace, and flood-plain complexes. Particular physical factors controlled by local position and by slope are mentioned in a former article (18).

Atmospheric factors vary locally in this region to a probably not very great extent, but even slight differences may be critical, as has been found by COOPER in the California chaparral. The factor of greatest influence upon plant life, and the one most readily measured, is the evaporating power of the air, the value of which represents the resultant of several contributing factors. Local distribution of evaporating power is believed to be controlled primarily by differences in topography, and secondarily by differences in vegetation-cover. That is to say, flatness of the land surface makes for comparative uniformity of exposure to wind and sun; hilliness causes diversity of exposure. Local water or wet-soil surfaces may lower evaporating power by contributing much water vapor to the air. Topography thus determines the *original* local distribution of evaporating power. This original local distribution is modified by vegetation-cover. In flat country the uniformity is changed. Low and open vegetation lowers evaporating power at the ground surface only slightly, but mesophytic closed forest lowers it very greatly (GATES 3, 4). In hilly country in not too humid climates the originally protected ravines and shaded or wind-sheltered slopes may develop mesophytic vegetation which still further lowers evaporation, while the originally exposed slopes and summits usually remain xerophytic. Thus, in the mountain-front region here considered, primary environmental differences

due to topography may rather thoroughly control vegetation distribution. In such cases the reaction of vegetation-cover upon local evaporation conditions may merely heighten the original topographically determined contrast between protected and exposed habitats. Topography governs local vegetation distribution through the mediative influence of a number of physical factors, of which evaporating power is one. Depending as it does upon several other factors, evaporation forms a convenient index of habitat, but is not in itself the basic controlling condition. For these reasons the writer has subordinated the influence of evaporating power upon local distribution to that of topography.

The sudden change of elevation at the mountain-front is a topographic condition affecting evaporating power. At many places the hogbacks, mesas, and outer slopes receive no direct sunlight during several hours before sunset, being shaded by the higher slopes immediately to the west. This contributes to the comparative mesophytism of certain mountain-front stations where the descent from foothills to plains is more than ordinarily abrupt.

Direction of exposure affects local atmospheric conditions and vegetation in many easily observed ways. Cloudiness and showers occur on summer afternoons much more frequently than in the mornings, as RAMALEY has noted. East-facing slopes are thus likely to be drier than west-facing slopes (the latter are less frequent east of the range-crest). As would be expected, the difference between north- and south-facing slopes is considerable, the latter being more exposed to sun and conditions favoring rapid evaporation, and with sparser, more xerophytic vegetation. In open parts of the foothills where slopes are quite gentle the north-facing slopes are not sufficiently sheltered from sun and wind to differ in vegetation from the south-facing slopes in any marked degree. Steep north slopes, or both sides of steep and narrow ravines which run down to the north, however, are quite mesophytic. In different parts of so large a territory the combinations of contrasting vegetation of north and south slopes would be expected to vary, and a few of them are listed herewith by way of illustration.

TABLE VI

EFFECTS OF DIRECTION OF SLOPE UPON LOCAL DISTRIBUTION

Locality	Vegetation of south-facing slope	Vegetation of north-facing slope
Poudre foothills	Grassland	Scattered rock pine, with more mesophytic vegetation infrequent
Foothills near Boulder	Grassland, rock pine, mixed shrub	*Pseudotsuga*, canyon forest, rock pine, mesophytic grassland
Poudre mountain-front	Grassland	*Cercocarpus*, with very scattered rock pines in rocky places; grassland in fine soil
Mountain-front near Boulder	Grassland, mostly	Grassland with rock pine, mixed shrub, etc.
South of Golden, mountain-front	Grassland and *Cercocarpus*	Grassland with scattered rock pine and mixed shrub
Perry Park	Oak and grassland	Rock pine and *Pseudotsuga*
Palmer Lake	Oak	*Pseudotsuga*
Southern mountain-front in general	Pinyon-cedar, dry grassland, and scattered oaks	Closer and taller oak growth with rock pines

FACTORS OTHER THAN PHYSICAL CONDITIONS OF HABITAT.—If the physical conditions which determine the habitat and all their interactions and variations were fully known, however, the local distribution of plant communities as observed would only partially be explained. Within even a very small part of the region studied correlations between physical habitats and vegetation-types must not be too closely drawn. The rock pine, for example, grows in any soil or on any slope; its presence or absence in any particular situation is not alone a matter of physical conditions there and then operative. Local distribution of vegetation-types in these partly unstable and locally very diverse situations depends also on at least three other sets of conditions: (1) range of toleration, in individual species or groups of species, of variation of physical conditions; (2) local historic factors, physical and vegetational, which have been operative in any given spot (these often cannot be determined); (3) accident of seed distribution and germination. For these reasons it seems best to characterize the vegetation-units, in most cases, from the vegetation itself rather than from habitat. There can be no question

that, in general, local variation of present physical conditions of the habitat governs to a considerable degree the distribution of plant communities, but the need of at least recognizing these other sets of factors should be emphasized. It must be further seen that, in the invasion of a new habitat, representatives from more than one plant community can be successful in establishing themselves, resulting in *mixed vegetation-types*. In fact, probably the greater part of the area studied is occupied by mixed associations or *mictia* (CLEMENTS). Even areas of established vegetation are usually open enough to permit the continual ecesis within them of new plant immigrants from quite different communities. This diversity is likely to be relatively enduring, for plant competition usually does not here operate to exclude all but a single type of dominants. The opposite relation between plants, which may be called accommodation, is as greatly in evidence. The control exerted by vegetation upon the physical environment is slight over the generally xerophytic mountain-front region.

A second factor contributing to the mixed effect is the frequent extremely local variability of physical conditions within the habitat. This might be called *mosaic variability*, and its effect a *mosaic mixture* of vegetation-types. The influence of large surface rocks partly imbedded in fine soil, allowing the growth of comparatively mesophytic plants in a rather constant interspersal with xerophytes over a considerable area, may be cited as an example.

Vegetation-types and their distribution

Since the plant communities have been described separately in the two articles preceding this, their systematic characterization here may be condensed very considerably. A tabular view of the communities, giving some idea of their general character and of their distribution in the main geographic divisions of the region studied, is shown in table VII.

Some of the more important features of the particular associations may now be noted. Details and references to other accounts may be found in the articles preceding. The general appearance of certain vegetation-types may be seen in fig. 17.

Lichen association.—Lichens, especially *Rinodina*, *Lecanora*, and *Parmelia conspersa*, partly cover the dry rock surfaces, especially granites in the foothills and the craggy outcrops and loose surface rocks of the mountain-front. Rock exposures are infrequent in the plains proper.

TABLE VII

CONSPECTUS OF ASSOCIATIONS

	Foothills	Mountain-front	Plains
Thallus vegetation	Lichen association	Lichen association	(Lichen association)
Grassland			
Extensive, climatic	Foothills grassland	Mixed short-grass	Short-grass
Local, edaphic			
Xerophytic	.....	Wheat-grass *Stipa-Aristida*	Wheat-grass *Stipa-Aristida*
Less xerophytic	Bunch-grass, plus a foothills element Mesophytic grassland	Bunch-grass Prairie-grass	Bunch-grass (Local, infrequent, prairie-grass-like communities)
	forest herb type meadow type	meadow type mixed type	(meadow type) (mixed type)
Primitive	Foothills primitive grassland *Artemisia frigida* consocies	Primitive grassland *Artemisia frigida* consocies Primitive bunch-grass	Primitive grassland *Artemisia-Gutierrezia* consocies Primitive bunch-grass
Shrub vegetation			
Xerophytic	*Cercocarpus* association	*Chrysothamnus-Sarcobatus* association *Cercocarpus* association	*Chrysothamnus-Sarcobatus* association
Xerophytic to mesophytic	Mixed shrub association *Arctostaphylos* *Ceanothus* association *Symphoricarpos*	Mixed shrub association *Symphoricarpos*	(Local) shrub communities) (*Symphoricarpos*)
Tree vegetation			
Coniferous			
Xerophytic	Pinyon-cedar association	Pinyon-cedar association	
Less xerophytic	Rock pine association	Rock pine association	
Relatively mesophytic	*Pseudotsuga* association	(*Pseudotsuga* association)	
Deciduous			
Xerophytic to mesophytic	Oak association *Populus-Salix* association	Oak association *Populus-Salix* association	*Populus-Salix* association
Relatively mesophytic	Canyon forest (Aspen association)	Canyon forest	

Associations with equivalent or similar representation in plains, mountain-front, and foothills areas are shown on the same horizontal line. Very local or poorly developed representation of a community in a particular zone is indicated by parentheses.

Mixed grasslands and short-grass.—The shallow-rooted short-grasses, *Boutcloua* and *Bulbilis*, dominate the compacted fine soil surface of most of the plains, as the well known short-grass association. *Boutcloua* alone, with admixture of plants of different physiological and geographic character, is the important element of dry

FIG. 17.—Distribution of vegetation in typical northern mountain-front area (outlet of Gregory Canyon, near Boulder): habitats and vegetation-types distinguishable are: 1, craggy exposure of Fountain sandstone; 2, rock talus, with beginning of primitive grassland; 3, stabilized slope with primitive grassland; 4, mixed grassland, in places approaching mesophytic type, mostly on northwest-facing slope; 5, more nearly xerophytic mixed grassland, with bunch-grass and ruderal elements on northeast-facing slope; 6, *Symphoricarpos;* 7, mixed shrub; 8, canyon forest; 9, *Populus angustifolia* stream-side community; 10, rock pine; 11, *Pseudotsuga*, in scattered growth, including rock pine and other components, on north-facing slopes of Green Mountain.

grassland in the débris-covered soil of the mesas and outwash-plains of the mountain-front (short-grass mixed association). The foothills mixed grassland, very similar to the mixed short-grass, is typical over the coarse surface of the granitic foothills.

Wheat-grass.—This taller but also shallow-rooted grass, *Agropyron Smithii*, dominates areas of loose clay in the mountain-front and plains. Its ecological character is not well understood.

Stipa-Aristida association.—These tufted xerophytic grasses of coarse soil occur frequently but not extensively, together or singly, with other rather deep-rooted plains xerophytes.

Bunch-grass.—Tufted perennial and deep-rooted grasses, depending on continued moisture, such as *Andropogon scoparius*, *A. furcatus*, *Sorghastrum nutans*, etc., are notable through most of the prairie region, almost absent in dry plains, but abundant in the rain belt of eastern Colorado; at the mountain-front and in the foothills, scatteringly in the north, but very frequent at the Platte-Arkansas divide and southward into New Mexico. In the foothills there are grasses of similar habit which mingle with the prairie bunch-grasses.

Mesophytic grasslands.—Mesophytic herbaceous growths are made up partly of prairie plants and partly of Rocky Mountain forest plants. The latter element is very considerable in occasional foothill ravines. Meadow growths of both foothills and mountain-front, in moist soil, with showy flowering plants like *Delphinium*, *Cerastium*, *Castilleja*, *Orthocarpus*, etc., are conspicuous in early summer, but not very frequent. The mountain-front in many places shows a mixed grassland much like that of eastern prairie, which has been called western prairie-grass. It has plants of the mixed short-grass, with components from bunch-grass and eastern prairie or forest border, with some foothills mesophytes, and a few plants characteristic of the mountain-front, like *Stipa viridula*.

Primitive grasslands.—Early stages of grassland developing in areas recently bared, or remaining for long in loose shifting slopes, are frequently seen. Prostrate plants with heavy taproots (rosette plants) are common. Gravel-slides in the foothills and dry stony crests of mesas, buttes, and ridges in the plains and mountain-front are the typical habitats. The *Boutcloua hirsuta*

and the *Artemisia frigida* consocies may be mentioned specially. The last is closely allied to the *Gutierrezia-Artemisia* association of the plains, very widespread, and continuing, at the expense of short-grass, with heavy grazing. In mountain-front and plains the primitive bunch-grass association, with *Panicum virgatum*, *Sporobolus cryptandrus*, *Stipa Vaseyi*, *Eriocoma*, etc., occupies sandy or loose-soil habitats recently disturbed.

Chrysothamnus-Sarcobatus association.—The shrubby composite, *Chrysothamnus* (rabbit-brush), and the chenopodiaceous greasewood occupy loose soil, mostly alkaline areas, on certain slopes in the mountain-front, and are particularly abundant in stream-bottoms in the southern plains.

Cercocarpus association.—Mountain-mahogany, of the rose family, is the only dominant in the open shrub growth of the mountain-front and outer foothills, in very dry exposed situations and usually stony soil. In the interstices between shrubs are plants of primitive grassland or mixed short-grass.

Mixed shrub association.—This is a heterogeneous assemblage of shrubs, ranging from xerophytic, like *Rhus trilobata*, to relatively mesophytic forms, like *Crataegus coloradensis*, in sheltered situations. The same species range through a variety of habitat conditions, and may form a community either as shrubs or trees. The mixed shrub grades into the canyon forest.

Arctostaphylos association.—The well known and widespread bearberry forms its characteristic mats in the foothills, mostly on compacted gravelly floors. It is more abundant in the upper foothills, in open places among the scattered pines. Its congener, *Juniperus communis sibirica*, is present but infrequent.

Ceanothus association.—*Ceanothus Fendleri* forms low matlike ground-cover in the lower foothills, similar to that of *Arctostaphylos*, though it is not evergreen, is of more southerly distribution, and ranges into drier and more exposed habitats. It favors the establishment of seedling mesophytes, and plays a part in revegetation of burned areas.

Symphoricarpos association.—The buckbrush, as it is called, occupies moist fine soil, and invades grassland in the mountain-front and foothills, as well as in the eastern prairie, in favorable

situations such as draws and seepage areas of slopes. It in turn is frequently displaced by taller woody vegetation.

Rock-pine association.—*Pinus scopulorum* is the important tree of the foothills. It ranges into very variable habitats, and is structurally variable in accordance. It forms infrequent close stands, but in most places is scattered, the ground between the trees being occupied by foothills mixed grassland, *Ceanothus*, *Arctostaphylos*, etc. It is frequent in rocky crests, etc., in the mountain-front, except in the south, where it is commonly replaced by pinyon. It extends very locally into the plains in broken country, on butte-crests, etc., and on the elevated Platte-Arkansas divide.

Pinyon-cedar association.—*Pinus edulis* and *Juniperus monosperma* are important xerophytic conifers of the southern mountain-front and lower foothills north to the Garden of the Gods, and extending into the southern plains on mesa-crests, canyon-walls, and bluffs of broad valleys. The soil is usually rocky or gravelly. The trees are low and rounded, and do not form a closed assemblage.

Pseudotsuga association.—*Pseudotsuga mucronata* forms the mesophytic or relatively mesophytic coniferous forest of the region, and is confined to sheltered ravines and steep north slopes in the foothills. It is infrequent at the mountain-front.

Oak association.—Small trees of the white-oak group, of uncertain taxonomic affinity, form dense copses or open woods in the lower foothills and in the mountain-front about as far north as Platte Canyon. In places grazing destroys the oak slowly and allows increase of grassland. The undergrowth of mesophytic oak areas is much like that of the canyon forest.

Populus-Salix association.—In stream-side areas of the foothills *Populus angustifolia* and 4 or 5 common willow species are frequent. Outside the mountains *Populus Sargentii*, and in the south *Populus Wislizeni*, replace the narrow-leaf cottonwood. Cottonwoods extend eastward into the plains for many miles along watercourses.

Canyon forest.—The deciduous trees of the foothill canyons and of ravines, etc., in the mountain-front, include *Alnus tenuifolia*, *Betula fontinalis* (these two common along mountain streams),

Amelanchier alnifolia, *Prunus pennsylvanica*, *P. americana*, *P. demissa*, *Crataegus coloradensis* et spp., *Robinia neomexicana* (in the south only), *Acer glabrum*, and *A. Negundo*, with a few others. A few shrubs are present and a variable undergrowth, with one typical aspect of *Viola canadensis* Rybd., *Hydrophyllum*, and *Galium*. *A Ligusticum* is very abundant in places.

Aspen association.—*Populus tremuloides* is restricted, in all but the highest parts of the foothills, to relatively mesophytic ravines. It does not come up abundantly following burning of the pine forest, as is true in the higher elevations and farther north.

Eastern Illinois State Normal School
Charleston, Ill.

LITERATURE CITED

1. Davis, W. M., The Colorado Front Range. Ann. Ass. Amer. Geog. 1:21–83. 1911.
2. Fenneman, N. M., Geology of the Boulder district, Colorado. U.S. Geol. Surv. Bull. 265. pp. 101. 1905.
3. Gates, F. C., The relation between evaporation and plant succession in a given area. Amer. Jour. Bot. 4:161–178. 1917.
4. Gleason, H. A., and Gates, F. C., A comparison of rates of evaporation in certain associations in central Illinois. Bot. Gaz. 53:478–491. 1912.
5. Johnson, W. D., The High Plains and their utilization. Ann. Repts. U.S. Geol. Surv. 21, part 4, pp. 599–741; 22, part 4, pp. 631–669. 1900, 1901.
6. Lee, W. T., The origin of the débris-covered mesas of Boulder, Colorado. Jour. Geol. 8:504–511. 1900.
7. Marvine, A. R. [The sedimentary rocks east of the Front Range (chap. ii, pp. 93–137, in Marvine's report)], in Hayden, F. V., Ann. Rept. U.S. Geol. and Geog. Surv. Terr. for 1873, embracing Colorado. 718 pp. Washington, 1874.
8. Ramaley, F., Plant zones in the Rocky Mountains of Colorado. Science 26:642–643. 1907.
9. ———, Botany of northeastern Larimer County, Colorado. Univ. Colo. Studies 5:119–131. 1908.
10. ———, Dry grassland of a high mountain park in northern Colorado. Plant World 19:249–270. 1916.
11. ———, Vascular plants of the Tolland region in Colorado. Univ. Colo. Studies 12:27–51. 1917.
12. Ramaley, F., Robbins, W. W., and Dodds, G. S., Studies in mesa and foothill vegetation, I. Univ. Colo. Studies 6:11–49. 1908.

13. Robbins, W. W., Climatology and vegetation in Colorado. Bot. Gaz. 49:256–280. 1910.
14. ———, Native vegetation and climate of Colorado in their relation to agriculture. Bull. 224, Agric. Exp. Sta. of the Colo. Agric. Coll. 56 pp. 1917.
15. Shantz, H. L., A study of the mesa region east of Pike's Peak: the *Boutcloua* formation. Bot. Gaz. 42:16–47, 179–207. 1906.
16. ———, Natural vegetation as an indicator of the capabilities of land for crop production in the Great Plains area. U.S. Dept. Agric., Bur. Pl. Industry, Bull. 201. pp. 100. 1911.
17. Vestal, A. G., Prairie vegetation of a mountain-front area in Colorado. Bot. Gaz. 58:377–400. 1914.
18. ———, Foothills vegetation in the Colorado Front Range. Bot. Gaz. 64:353–385. 1917.
19. Ward, R. De C., Rainfall types of the United States. Geog. Review 4:131–144. 1917.
20. Weaver, J. E., Evaporation and plant succession in southeastern Washington and adjacent Idaho. Plant World 17:273–294. 1914.

ON NITRIFICATION[1]

III. THE ISOLATION AND DESCRIPTION OF THE NITRITE FERMENT[2]

AUGUSTO BONAZZI

(WITH PLATE XIV)

Introduction

As a part of the physiological investigations upon nitrification carried on in this laboratory, the isolation of pure cultures of the nitrite-forming organisms became a necessity. Since difficulties in the isolation of these organisms have been encountered by many workers in the field of soil bacteriology, and since no accurate description of the organisms of nitrification from North American soils has been published as yet, it was deemed advisable to describe the organisms responsible for the formation of nitrites in the Ohio soils and the methods used in their isolation.

The following contribution, which is the result of a long period of study, is here given to describe an organism, capable of forming nitrites from ammonia, isolated in a pure state from Wooster soil after many unsatisfactory attempts.

Historical

In 1890 JORDAN and RICHARDS (4) in Massachusetts stated that they had isolated a nitrifying organism which was capable of oxidizing ammonia salts to nitrates when grown in solutions free of organic matter. The dilution method was the one relied upon by these investigators for obtaining pure cultures. Their description of this nitrifier is here reproduced.

[1] Contribution from the Laboratory of Soil Biology, Ohio Agricultural Experiment Station.

[2] The first paper of this series dealing with the subject of nitrification appeared under the title "Preliminary observations" in Ohio Agric. Exper. Sta. Technical Bull. 7. 1915. The second paper under the title "Intensive nitrite formation in solution" appeared in Jour. Bacteriology 1918. Thanks are due Dr. E. R. ALLEN, in whose laboratory this work was undertaken, for kind advice and criticism.

The bacilli are short, of a slightly oval shape, and vary from 1.1 μ to 1.7 μ in length; they are about 0.8–0.9 μ broad. They are grouped characteristically in irregular clumps and are held together by a jelly-like material. Each aggregation is indeed a typical zooglea. The aggregations of bacteria were found chiefly in the bottom of the flasks, as was also the case with the organism described by WINOGRADSKY. . . . . On one important point there appears to be a difference between our results and those reached by the above mentioned investigators. The organism discovered by them oxidizes ammonia to nitrite, but carries it no further. Our flasks give complete oxidation to nitrate. . . . . We are not even prepared to say that there may not have been a mixture of two or more species in our flasks, all agreeing closely in morphological characters, and in giving no growth on gelatin, but differing in important physiological respects.

This statement makes it quite clear that probably their cultures were a mixture of the two forms isolated and separated by WINOGRADSKY (11). No lengthy description of the organisms isolated by the Russian investigator is here necessary, and a summary review of his findings (12) will suffice. The nitrite-forming organisms from different parts of the world were divided by WINOGRADSKY in two genera and several species as follows: *Nitrosomonas* (*N. europeae* and *N. javaniensis*), from the Orient; and *Nitrosococcus*, from the Occident. *Nitrosomonas* received by far the greatest attention from WINOGRADSKY, while *Nitrosococcus*, which is most important to American economy, he only incompletely described. The soils from which WINOGRADSKY isolated his *Nitrosococcus* were from Campinas (Brazil), Quito (Ecuador), and Melbourne (Australia).

Samples of North American soils were not studied, and it is surprising that no description of organisms from such soils has been attempted since the time of the discovery of the active agents of nitrification. It is true that JORDAN and RICHARDS gave a description of the sewage organisms they were working with, but their description does not conform to the one given by WINOGRADSKY of the South American organisms. It is evident, therefore, that, were it only from a geobotanical standpoint, the description of the organisms from the Northern Hemisphere presents some importance.[3]

[3] The investigations reported by OWEN, WM. L. (The effect of carbonates upon nitrification. Georgia Exper. Sta. Bull. 81. 1–42. 1908), should not be overlooked in this connection. Unfortunately it must be admitted that the photographic reproductions of the organisms which he found in his flasks remind us only of a mixture of

The organism from Quito soils had, according to its discoverer, the following characters: a large coccus 1.5–1.7 μ in diameter, appearing larger in the living state than in the stained preparations. According to WINOGRADSKY this was probably due to a thick gelatinous membrane which did not stain or become invisible on desiccation. Typical zooglea, as those of *Nitrosomonas*, were never found, and the motile stage was never observed in liquid cultures of this organism. Corresponding to this behavior in solution, the colonies on silica jelly were only of one form, and were never seen to simulate the "clair colonies" of the *Nitrosomonas*. Surface colonies had the appearance of a yellowish liquid, and like the deep colonies were also made up of free cells. The organisms from Campinas and Melbourne showed the same characters, but were only different in size from the Quito organism, the Campinas coccus attaining a size of 2 μ in diameter, the Melbourne organism not quite 1.5 μ in diameter (a little smaller than the other two, WINOGRADSKY **12, 13**).

From this cursory review of the descriptions of the organisms it is evident that little is known, especially with regard to their cultural characters. A footnote in WINOGRADSKY'S paper (**12**) describes a peculiar linking of the cells of the Campinas organism which the author only observed once, and was not therefore in a position to study carefully. It should be mentioned also that this same Campinas organism gave a "trouble sans motilité" which WINOGRADSKY could not explain.

Criterion of purity

One difficulty encountered in the present work was the establishment of a criterion of purity, a standard sufficiently accurate to allow its general acceptance. The words "pure culture" in bacteriological literature are used to indicate such a culture as is made up completely or almost entirely of cells of the same type,

various ubiquitous bacteria, which were not active in nitrification. The actual nitrite and nitrate production in the cultures studied by OWEN was so slight, to justify their being considered non-nitrifying cultures, that this fact, together with the heterogeneous bacterial contents, points to the possibility that he might not have possessed pure cultures of nitrifying organisms.

showing within the limits of individual variations in the same species the same physiological and morphological characters. Although theoretically a "pure culture" is that culture originated monocytogenetically, in practice it is seldom obtained. True pure cultures are only possible when the development of one single cell into a colony is controlled directly by microscopical observation; in ordinary technic this is not done. Although these requisites are necessarily of the same importance when "pure cultures" of nitrite- and nitrate-producers are desired, the term "pure culture" of nitrifying bacteria does not convey this same criterion of "absolute purity."

The simple fact that WINOGRADSKY found the nitrate- and nitrite-formers of different lands to possess in common the character of not growing in bouillon, in spite of their morphological differences, and that he made of this a criterion of purity, is enough to show the misleading interpretation given to the term "pure culture." The criterion of purity formulated by WINOGRADSKY (12) is as follows: "Introduce a loopfull of a nitrified culture in ordinary bouillon and keep at 30° C. during 10 days; at the end of this time the bouillon must not show turbidity. The purity of the nitrifying organisms is then proven."

That this criterion of purity is rather indefinite is shown by the following statement from JORDAN and RICHARDS, who, discussing the purity of some of their cultures, state that "we are not even prepared to say that there may not have been a mixture of two or more species in our flasks, all agreeing closely in morphological characters, and in giving no growth on gelatin, but differing in important physiological respects." OMELIANSKI (6) states that "after renewed ammonia additions it is necessary to control the purity by microscopical examinations as well as reinoculation in bouillon."

Since WIMMER (9) found a culture of nitrifying organisms to prove pure on bouillon of one reaction, while this same culture proved impure when tested on bouillon of a more alkaline reaction, the author has adopted in his work with nitrite-formers the following cardinal points which form the basis for his criterion of the purity of a culture: (1) the culture must be in full nitrification

before any attempt to determine the purity by the bouillon method is made; (2) the bouillon used in testing for purity should be of an alkaline reaction; better results would be obtained if the testing were made in bouillon of different reactions; (3) the time limit necessary for absolute reliability for the growth in bouillon should be fixed at 10 or more days; (4) the inoculum used in testing for purity by the bouillon method should be as large as permissible by ordinary technic; (5) microscopical examination must reveal a picture uniform within the limits of individual morphological variation in the species and within the different phases of the same organisms, provided such phases are established as correct.

A study of the growth of different organisms on the same medium and the description thereof is often relied upon as a diagnostic method. At best this method is unsatisfactory and of limited application. This is plainly shown by the fact that we cannot attempt to compare, on the same basis, the ordinary saprophytic and parasitic organisms with such organisms as will not grow on the media used in ordinary laboratory practice. The known organisms of the latter type are not numerous at present, but doubtless they are quite abundant in nature. The nitrite-formers of WINOGRADSKY are to be classified among the latter, together with the not less peculiar *B. oligocarbophilus*, *B. pantothropus*, and some organisms of the oligonitrophilus, sulphur, and iron groups. In the case of these organisms a study of their morphology in a few special media will lead to more accurate and reliable results than any attempt made to grow them in bouillon, gelatin, or agar of the ordinary composition.

Having presented the description of the South American nitrite-formers, and the criteria of purity followed in the present work, a description of the organism isolated to comply with these rules, from a North American soil, will be given after a discussion of the isolation technic.

Experimental

After establishing nitrification in solution by means of a small soil inoculum, the growth of the organism concerned was stimulated by continued additions of new doses of ammonium sulphate, new

ammoniacal salt being added only if the culture showed no ammonia reaction when tested with Nessler's reagent. From these enrichment cultures new cultures were started in solution with small inoculi. The culture solution used in all this work was the one recommended by OMELIANSKI (6), of the following composition: H_2O, 1000 cc.; $FeSO_4$, 0.4 gm.; $MgSO_4$, 0.5 gm.; K_2HPO_4, 1.0 gm.; NaCl, 2.0 gm.; $(NH_4)_2SO_4$, 2.0 gm.

Filtration of the solution was deemed unnecessary, since the addition of $MgCO_3$ milk after sterilization would make the practice useless. Growth was not impaired by preliminary filtration of the medium. After several transfers in solution, attempts were made at the isolation of pure cultures by plating in ammonium sulphate washed agar. Two consecutive platings in this medium

TABLE I

Soil	0	Silica jelly plates	63
Solution	19	Solution	66
Solution	24	Silica jelly plates	71
Washed agar plates	34	Solution	76
Washed agar slants	40	Solution	84
Washed agar plates	50	Solution	86
Solution	59		

gave cultures which did not fulfil the requirements of the "criteria of purity." It was only after 3 consecutive platings in a silicic acid jelly medium (6) that cultures were obtained which proved pure to the criteria. The genealogic succession of the cultures is shown in table I, where the series number accompanies the description of the medium used in each generation studied.

It was only a culture of series 86 in solution that, when inoculated in bouillon of a +1 per cent and a −1 per cent reaction, gave no growth whatsoever, either before or after the 10-day period had elapsed. Microscopically no foreign forms were seen, and a perfectly pure culture was indicated by this test. Unfortunately, after this culture was obtained it was observed that its action was relatively slow, and in subsequent transfers the inoculi used were very large, 1 or 2 cc. Notwithstanding these precautions, after the cultures had been transferred once or twice again they

gave no more the characteristic nitrite formation. The genealogic succession is represented in table II.

Tests made in bouillon (of a + and − reaction) proved cultures of series 90 and series 94 to be absolutely pure. Cultures of series 86 were inoculated with 1 cc. of the mother culture, those of series 88 with 1 cc., those of series 90 with 1.5 cc., those of series 94 with 2.5 cc., and those of series 101 with one large loop of such a shape that it contained 2–3 drops of solution. The reaction of cultures from series 94 was questionable, and microscopic examination of some cultures of this series showed the organism to be very slow growing, so slow in fact as to allow a contamination to enter during the manipulations subsequent to the first test for purity. The nitrite formation of culture 101 was nil after nearly a month, and its cellular contents nil. That the loop used in this

TABLE II

Solution	86	Solution	94
Solution	88	Solution	101
Solution	90		

case was large enough for a successful inoculation, provided the mother cultures were growing well, is proved by the thousand other successful inoculations by this method.

The characters of the organisms isolated during the preceding series of generations are described later. Although these organisms in the pure state tended to lose their nitrifying power when cultivated in solutions at rest, by cultivating them in ignited soil, to which the ordinary OMELIANSKI solution and magnesium carbonate were added, it was possible to stimulate their action considerably. The cultivation of these same organisms in solutions undergoing a slow rotary movement and constant aëration proved them to possess a very strong nitrifying power. Organisms which were slowly losing their power of nitrification in the ordinary laboratory condition, as they approached a state of purity, were soon made to increase this power, up to an intensive nitrification, by appropriate means (BONAZZI 2).

Cultures in solution

Macroscopic examination of young solution cultures reveals no indication of bacterial growth, and it is not until much ammonia has been oxidized to nitrite that any macroscopic growth is apparent. By observing the bottom of the culture flask 30–40 days' old an inconspicuous slimy deposit is visible which is easily dispersed by shaking. Before this point is reached no clouding or movement of the solution is visible, such as the "trouble" imparted to the solution by the "monad stage" of the European organisms. In fact no distinction can be drawn between the inoculated and non-inoculated flasks incubated for the same period of time. This similarity is maintained throughout the life of cultures which have been kept in this laboratory for over 2 months. No surface growth is visible, even when the cultures have attained very old age. Two cultures, which had nitrified 74.41 and 48.10 mg. of ammoniacal nitrogen while at rest, showed no surface growth whatever. No motility can be observed in the cultures, and this is in conformity with the behavior of the South American cultures described by WINOGRADSKY. In all the work with solutions at rest we have adopted the use of 20 or 25 cc. of solution in 250 or 300 cc. Erlenmeyer flasks, since this depth of solution furnishes a relatively good aëration.

Cultures on solid media

Several solid media have been tried in this laboratory in the hope that a satisfactory method could be found which would be advantageous to the speedy growth of the organism of "nitrosofermentation." Among others there were tried the paper pad method and the gypsum block method of OMELIANSKI (6), the magnesium carbonate block method of PEROTTI (7), the magnesium carbonate and ammonium-magnesium-phosphate block method of MAKRINOFF (5), the ammonium sulphate washed agar method of BEIJERINCK (1),[4] the silicic acid jelly method of STEVENS and

[4] The method of BEIJERINCK was also modified so that the washed agar only came in contact with the salts a few minutes before inoculation. This was accomplished by incorporating, just before plating, the necessary quantity of salts dissolved in 5 cc. of water with 5 cc. of a melted washed agar jelly, mixing thoroughly, inoculating, and plating. The action of the salts on the agar at high temperatures was thereby avoided.

TEMPLE (8), and the silicic acid jelly method of WINOGRADSKY (10). By far the best and most reliable results were obtained with WINOGRADSKY'S silicic acid method, which has now been adopted in this laboratory as the best among all those tried. When an impure enriched culture is inoculated in silicic acid jelly plates superficially, very little visible growth takes place. The point of inoculation only assumes a perlaceous aspect and does not change with age.

When the inoculum is incorporated with the nutritive jelly and incubated at 30° C., no growth is visible before 11 or 12 days have elapsed from the time of inoculation. It is only after this period that small colonies are visible to a magnification of 75 diameters, barely distinguishable in the thick mass of crystals formed in the plate. After the lapse of a few days more the colonies reach a size of $\pm 224 \times 160\,\mu$; when observed by transmitted light they have the appearance of small yellowish masses surrounded by a colorless halo, which is due to the solution of the $MgCO_3$. This characteristic may be utilized as a means of differentiation in the identification of the colonies (fig. 1). The colonies have at first diffused outlines, and slowly take on a more definite form, their appearance being well shown in figs. 2 and 3. They never assume a hard consistency, remaining always soft. Colonies of 1 mm. diameter have been obtained by renewing the $(NH_4)_2SO_4$ in the plates when necessary, by the method recommended by OMELIANSKI.

Hanging drop cultures

Hanging drop cultures show no motility, even when the material is taken from mother cultures which are undergoing at the time a strong nitrification (figs. 7 and 8). Material prepared for microscopical observation from cultures undergoing intensive nitrification on a klinostat (BONAZZI 2) only showed a slow vibration of the cells, easily ascribable to Brownian movement.

Microscopical aspect of organism

In the beginning of the investigation the solution cultures were searched for any growth which might resemble the typical zooglea or the typical monad forms described by WINOGRADSKY

for the organisms of the Old World. Failing in the search, attempts were made to find any organism which, by its constant abundance in the cultures, might prove to be the principal type. On account of the slow growth of the organisms of nitrosofermentation in the ordinary laboratory conditions, it was not until nearly pure cultures were obtained that I observed, mixed with and imbedded in the salt deposit, a large coccus form, much resembling the one described by WINOGRADSKY as peculiar to South American soils. The strong "ferment power" of this organism had aided it in escaping attention in impure cultures. These megalococci are shown in fig. 6. They are large, $\pm 1.25\,\mu$ in diameter, and of a slightly irregular roundish form. Some are occasionally found which appear to have a triangular section, but closer observation reveals them to be clumps of smaller cells arranged so as to simulate a tetrahedron.

The microscopical examination of material from cultures in full nitrification, answering to the criterion of purity, is the most instructive. Fig. 5 shows the appearance of the megalococci at this time; they are composed of a thick gelatinous mass in which are imbedded small granules. Very often seemingly bipolated bodies are imbedded in this jelly, but on closer examination these polar bodies appear to be, not small cocci, but diplococci, the true living active units (figs. 4, 5). Material from intensive cultures, observed fresh in strong Meissner solution, showed these structures very plainly stained differentially and larger than in the dried preparations, since on drying the gelatinous mass seems to lose its thick structure. This gelatinous coat has a thickness that equals the diameter of the cells imbedded in it, and it takes on a bluish tinge in iodine, while the imbedded cells stain golden yellow in the same reagent.

When the cultures are in full and strong nitrification, the megalococci give rise to the small cocci which we will name β. The latter are clearly shown in fig. 5. These small β forms, which were at first imbedded in a thick gelatinous mass, forming the large α cocci, are set free and begin independent life, leaving the empty sheaths which are occasionally to be seen in stained preparations. Some of the β forms have been observed to take up a

gelatinous coating and revert to megalococci. This cycle recalls a little the cycle followed by the organisms of Java soils, as related by WINOGRADSKY (12, figs. 10, 12).

The staining methods adopted in this study are similar to the methods recommended by WINOGRADSKY. Although several of the ordinary staining solutions were tried, none seemed to give as good and clear pictures as the malachite-green and gentian-violet method. The salts, which are generally placed on the slide with the bacterial preparations, especially when solutions are the origin of the material studied, are not stained by this method. The technic is as follows: The coverglass preparation, flame fixed, is mordanted for 1 minute in the cold with a 0.25 per cent solution of malachite-green in distilled water, washed with cold water, and stained cold with a 0.25 per cent water solution of gentian-violet for 1 more minute. Washing is then done rapidly with water previously heated to 50–60° C. This washing takes out any coloration of the salts which might cloud the microscopic field. Preparations are thus obtained which stain the jelly of the megalococci a deep purple and the small cocci of the β type a purple-black color. Treatment with acid is not necessary to dissolve the salt formations. In the fresh unstained state the cells are not easily visible, and a search for them often proves unsuccessful. The color differentiation mentioned, obtained in Meissner solution, constitutes what seems to be the best and most reliable one for a study of the organisms in hanging drop preparations.

Temperature relations

The thermal death point of the organism studied was found to lie between 50 and 55° C., when the vitality of the organism, after heating 5.5 minutes at the required temperature, was tested at rest in OMELIANSKI'S solution containing basic magnesium carbonate. An additional study of the resistance of the organism to heat was also made. One cc. portions of a strong nitrifying culture were placed in sterile vials, heated at given temperature for given lengths of time, and then quickly cooled in stone cold water, the depth of the solution layer being 4–5 mm. over a surface of ± 2.8 cm^2. Heating was done in ovens, and tests for

the vitality of the cultures were made by inoculating all the heated material in large test-tubes containing 25 gm. of sterile coarse ignited soil and $MgCO_3$ kept in a slanting position by 10 cc. of sterile OMELIANSKI solution. Duplicates were run for each treatment. When the cultures were tested for the formation of nitrite after 12 days of incubation at 30° C., the results given in table III were obtained.

Although the duration of incubation was not very long, the nature of the medium was such as to allow complete nitrification in all cases where full inoculum was used. The fact that a heating of 40 minutes at 50° C., a heating of 10 minutes at 55° C.,

TABLE III*

Temperature of heating	Time of heating in minutes					
	2	5	10	20	40	60
45° C.	+	+	+	+	+	+
50° C.	+	+	+	+	o	
55° C.	+	+	?			
62–65° C.	+	o				

*The + indicates a strong nitrite formation, ? a questionable nitrite formation, and o no nitrite formation.

and a heating of 5 minutes at 62–65° C. gave questionable or negative nitrification, while all other cultures gave complete nitrification, is very significant.

BOULLANGER and MASSOL (3) found the thermal death point of the organism of nitrosofermentation isolated in their work to lie between 45° and 50° C. when the heating continued for 5 minutes. It is seen, therefore, that the organism isolated from Wooster soils resists higher temperatures and longer periods of heating than the one which constituted the object of their studies. The difference in apparatus used might lead to slight variations in the T.D.P. determinations.

The incubation of all the cultures of nitrite-formers cultivated in this laboratory has been done at 28–30° C. At this temperature cultures were obtained which nitrified as much as 8.04 mg. of ammoniacal nitrogen in 26 days of incubation at rest.

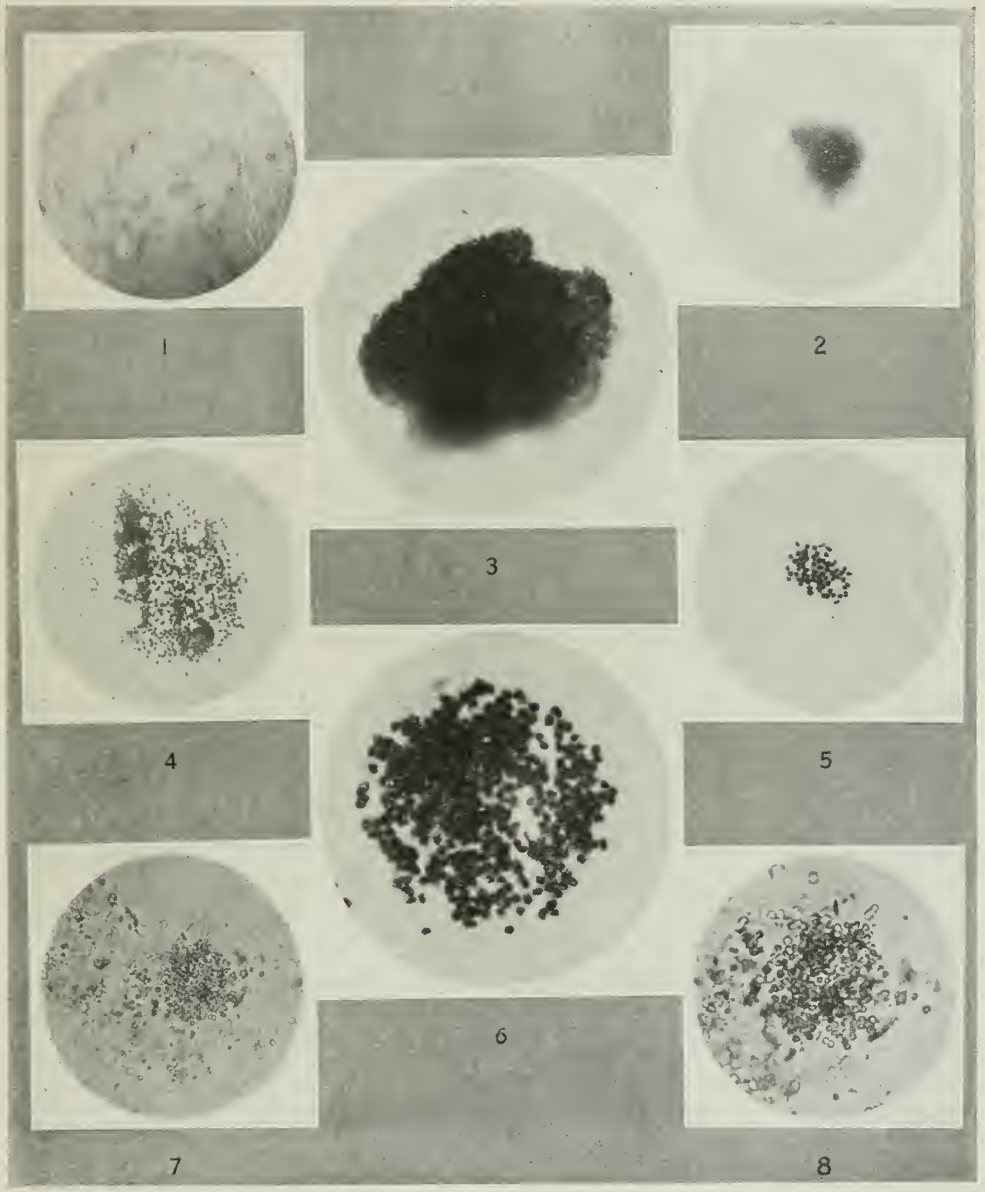
Discussion

The results of this study on the morphology of the organisms of nitrosofermentation, active in the North American soils, are not easily reconciled with the results obtained 28 years ago by JORDAN and RICHARDS. The discrepancy between the results is undoubtedly to be ascribed to two reasons: (1) incomplete description of the organisms isolated from sewage in Massachusetts, and (2) the probable presence in those cultures of organisms capable of transforming nitrites to nitrates. The possibility of a mixed culture was admitted by JORDAN and RICHARDS on the basis of the absence of nitrite formation during nitrification.

The resemblance of the megalococcus isolated by WINOGRADSKY from the South American soils to the megalococcus for the North American soils is quite striking. Excepting the slight difference in size existing between the two forms, all other characters are common to both. The variation in the life cycle of the two organisms is probably only apparent, since the true cocci, which have been named β by the author, might have escaped WINOGRADSKY'S attention. It is to be regretted that the Russian investigator was not able to study the nitroso-organisms from the New World as thoroughly as he did those from the Old World, and that he was obliged to publish only an abridged description of the former types. In the present work an attempt was made at the completion of his work, and results have been reached which corroborate the postulations to be made from his findings, in such a way filling a gap in our knowledge of the distribution of the nitrite-forming organisms. That the organism isolated in Wooster can be classed as a species of the genus *Nitrosococcus* seems to be justified by these findings. The comparative study of the species of the genus *Nitrosococcus* will have to be deferred to a time when a careful study will be possible of the organisms of nitrosofermentation in South America and Australia.

LITERATURE CITED

1. BEIJERINCK, M. W., Kulturversuche mit Amöben auf festen substrate. Centr. Bakt. I. 19:257–267. 1896.
2. BONAZZI, A., On nitrification. II. Intensive nitrite formation in solution. Jour. Bacteriology 4:43–60. 1919.



BONAZZI on NITRIFICATION

3. Boullanger, E., and Massol, L., Études sur les microbes nitrificateurs. Ann. Inst. Past. 17:492–515. 1903.
4. Jordan, E. O., and Richards, Ellen H., Investigations upon nitrification and the nitrifying organism. Experimental investigations by the State Board of Health of Massachusetts, etc. Part II. Report on water supply and sewerage, 1888–1890. pp. 865–881. 1890.
5. Makrinoff, J., Magnesia-Gipsplatten und Magnesia-Platten mit organischer Substanz als sehr geeignetes festes Substrat für die Kultur der Nitrifikationsorganismen. Centr. Bakt. II. 24:415–423. 1909.
6. Omelianski, V., Über die Isolierung der Nitrifikationsmikroben aus dem Erdboden. Centr. Bakt. II. 5:537–549. 1899.
7. Perotti, R., Di una modificazione al metodo d'isolamento dei microorganismi della nitrificazione. Atti. R. Acad. Lincei V. 14:228–231. 1905.
8. Stevens, F. L., and Temple, J. C., A convenient mode of preparing silicate jelly. Centr. Bakt. II. 21:84–87. 1908.
9. Wimmer, G., Beitrag zur Kenntniss der Nitrifikationsbakterien. Zeitsch. Hyg. 48:135–174. 1904.
10. Winogradsky, S., Recherches sur les organismes de la nitrification. Ann. Inst. Past. 5:92–100. 1891.
11. ———, Recherches sur les organismes de la nitrification. Ann. Inst. Past. 5:577–616. 1891.
12. ———, Contribution à la morphologie de la nitrification. Arch. Sci. Biol. St. Petersburg 1:87–137. 1892.
13. ———, Die Nitrifikation. Handbuch der Technischen Mykologie. 3:132–181. 1906.

EXPLANATION OF PLATE XIV

The photomicrographs were made with the kind assistance of Mr. Victor Dye, photographer of the Station.

Fig. 1.—Portion of silica jelly plate, showing colonies of *Nitrosococcus* after several additions of ammonium sulphate; solubilization of magnesium carbonate in vicinity of colonies very evident; ×1.5.

Fig. 2.—Colony of *Nitrosococcus* on silica jelly plate, showing loosely granular structure, as seen against a source of light; ×77.

Fig. 3.—Another colony on silica jelly; ×175.

Fig. 4.—*Nitrosococcus* from culture undergoing intensive nitrification, showing lack of zooglea formation; stained by malachite-green–gentian-violet method of Winogradsky; ×820.

Fig. 5.—*Nitrosococcus* from culture undergoing intensive nitrification, showing small β forms imbedded in thick gelatinous membrane; cocci and diplococci; stained by malachite-green–gentian-violet method of Winogradsky; ×820.

Fig. 6.—*Nitrosococcus* from culture undergoing intensive nitrification, showing typical megalococci; in some cases the small forms are seen appearing in thick gelatinous membrane, stained by malachite-green–gentian-violet method of Winogradsky; ×1600.

Fig. 7.—*Nitrosococcus* in hanging drop preparation, stained with Meissner's solution; ×740.

Fig. 8.—*Nitrosococcus*, same as no. 7; ×1200.

POLYXYLIC STEM OF CYCAS MEDIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 252

WARD L. MILLER

(WITH ELEVEN FIGURES)

The question which lends particular interest to the polyxylic situation in 4 of the cycad genera (*Cycas*, *Macrozamia*, *Encephalartos*, and *Bowenia*) is whether or not the separate, concentric, vascular cylinders all originate with the differentiation of procambium strands in the plerome cylinder, and whether or not true protoxylem and protophloem are formed in every or in any instance. If protoxylem and protophloem are formed, are they or are they not orthodox in their detailed structures, and what is their condition in the older parts of the stem?

It is the purpose of this investigation to discover the exact origin and behavior of the different cambiums which develop the separate vascular cylinders, and to formulate some definite conclusions regarding the vascular elements which will leave the matter of this unusual method of secondary growth more clearly understood.

Historical

Although research among the cycads has been comparatively limited by lack of suitable material for study, several accounts have been published which deal more or less directly with the present problem. During an investigation of *Cycas siamensis* in 1885, CONSTANTIN and MOROT[1] concluded that the cambium of each supernumerary zone, laid down outside the first or normal cylinder, originated in the pericycle of the cylinder next inside.

In 1896 WORDSELL[2] published an account of the polyxylic stem of *Macrozamia*. In this particular genus he found the corti-

[1] CONSTANTIN, J., and MOROT, L., Sur l'origine des faisceaux libéro-ligneux supernuméraires dans la tige des Cycadées. Bull. Soc. Bot. France 32:173. 1885.

[2] WORDSELL, W. C., Anatomy of stem of *Macrozamia* compared with that of other genera of Cycadeae. Ann. Botany 10:601–620. 1896.

cal cylinders diminishing in the thickness of their wood and phloem as they neared the tip of the stem, until finally they disappeared entirely, the outermost disappearing first and the innermost last. Protoxylem, at least of the spiral kind, he found to be entirely obliterated in the normal cylinder, although it seems to have been of frequent occurrence in the leaf traces, where the crushing pressure of thickening cells was less effective. He mentions no protoxylem in connection with the supernumerary cylinders, either as to its presence or absence, nor in connection with leaf traces coming from these cylinders.

SCOTT'S[3] work in 1897 led him to the conclusion that the polyxylic habit was a derivation from the habit of ancient stems among the Cycadofilicales which developed layers of concentric bundles, for example, *Medullosa*.

WORDSELL[4] again in 1900 published an account of the seedling stem structure in *Bowenia*. There he found beginnings of a supernumerary vascular cylinder outside the normal one. Hints of concentric bundles, which he found in *Bowenia* and earlier in *Macrozamia*, led him to agree with SCOTT in the idea of the phylogenetic origin of supernumerary cylinders.

COULTER and CHAMBERLAIN[5] published in 1910 a summary of previous investigation pertaining to the vascular anatomy of cycad stems, and in addition gave a short description of the gross topography of the polyxylic habit.

JEFFREY'S[6] work, published in 1917, is the most recent account of this cycad peculiarity. To him also it is apparent that supernumerary cylinders arise in the pericycle. He objects, however, to SCOTT'S conclusions in regard to the phylogenetic origin of these vascular cylinders; he believes rather that they are a result of an ancient climbing habit of the stem. Such situations, he says, are frequent in numerous climbing stems of the present time, stems of both gymnosperms and dicotyledonous angiosperms.

[3] SCOTT, D. H., The anatomical characters presented by the peduncles of Cycadaceae. Ann. Botany 11:399–419. 1897.

[4] WORDSELL, W. C., The anatomical structure of *Bowenia spectabilis*. Ann. Botany 14:159–160. 1900.

[5] COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. 1910.

[6] JEFFREY, E. C., The anatomy of woody plants. 1917.

The foregoing brief account of previous research, touching upon the problem at hand, gives a foundation upon which to work and from which to develop further lines of investigation.

Material and methods

Material used in the study of the problem was collected by Dr. CHAMBERLAIN near Rockhampton in Queensland, Australia. I take this occasion to express my appreciation of his generosity in giving up material from his own private collection for my study, and of his helpful suggestions during the investigation. The plant collected by CHAMBERLAIN was about 3 m. in height, as it occurred in nature, and bore at its tip a cluster of megasporophylls surrounded by a crown of foliage leaves. Two pieces were taken from the stem, one at the apex and one near the base. The former piece was the entire tip, including the upper 6 or 8 inches of the axis, together with foliage leaves and megasporophylls; the latter piece was a cross-section of the stem at a height of less than a foot above the soil, and was cut with a thickness of about 3 inches. Both pieces were put into formalin, where they have remained since the time of their collection in November 1911.

Pieces of the stem were thoroughly washed in water and then allowed to stand in 50 per cent hydrofluoric acid for a period of 4 weeks. Following this treatment, methods were employed which were based upon the fundamentals of technic as published by CHAMBERLAIN[7] in 1916. Such variations in these principles as were used grew out of the kindly suggestions of Miss LANGDON of this laboratory. To her my thanks are given for her valued assistance.

Investigation

GROSS TOPOGRAPHY

As would be expected, the pith of this specimen is relatively large. Its diameter at the stem base measures 5.3 cm., whereas the diameter of the entire stem at the base is only 20 cm. At the tip, where the gross diameter is 17.8 cm., the pith has a diam-

[7] CHAMBERLAIN, C. J., Methods of plant histology. 1916.

eter of 5.8 cm. There is practically no tapering to the columnar trunk excepting at the very apex, while the pith actually increases in diameter as it nears the apex, until it finally merges into the plerome cylinder (fig. 1).

At the base of the stem there are 3 separate and distinct vascular cylinders, the normal one nearest the pith and the 2 cortical ones developed concentrically about the normal. In previous accounts the first cortical cylinder has been reported to have a development of xylem and phloem equal to that of the normal cylinder, while the second and succeeding cortical cylinders diminish successively in that development toward the periphery of the stem. I find in this stem, however, that, near the base, the first cortical cylinder has a greater radial extent of xylem and phloem than has the normal one, while the second is about the equal of the normal, thus beginning the successive decrease of xylem and phloem development which would likely be continued further if other vascular cylinders were present (fig. 2). In each cylinder the xylem elements are of greater radial extent than phloem elements, the former occupying approximately three-fifths of the radial extent of the entire cylinder.

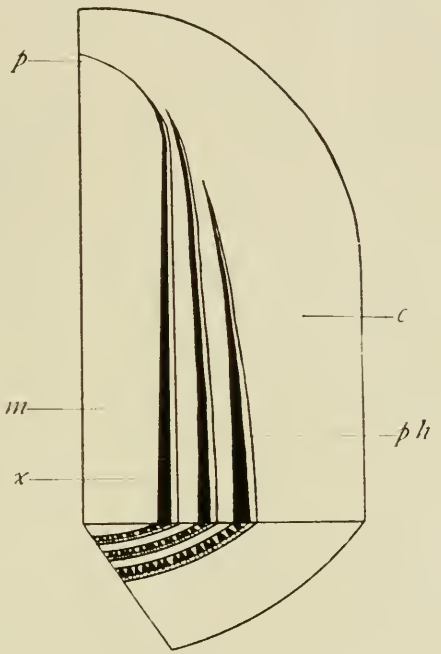


FIG. 1.—*Cycas media:* showing radial and transverse aspects of polyxylic stem near tip; *m*, pith; *p*, plerome; *x*, xylem; *ph*, phloem; *c*, cortex.

At the stem apex only 2 vascular cylinders are evident (fig. 3). Here the normal cylinder is seen to have xylem and phloem of slightly less radial extent than it has near the stem base, while

the first cortical cylinder has decreased so much in its dimensions that it is barely visible to the unaided eye. Furthermore, the latter occurs, not as a continuous cylinder, but rather as a cortical layer of separate, broad, and short bundles which are distinctly collateral. The outermost cortical cylinder entirely disappeared before reaching the height at which the section was taken. This

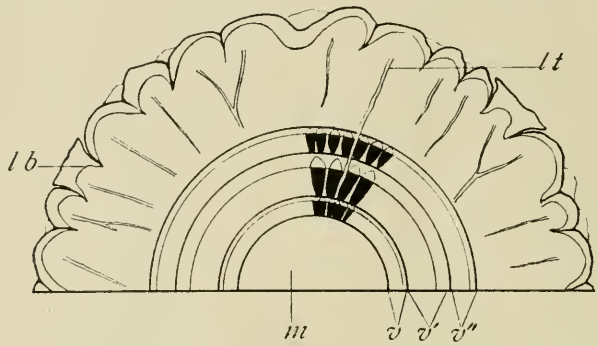


FIG. 2.—*Cycas media:* showing gross topography of transverse section of stem near base; *v*, *v'*, *v''*, 3 distinct vascular cylinders; *m*, pith; *lt*, leaf traces; *lb*, leaf bases.

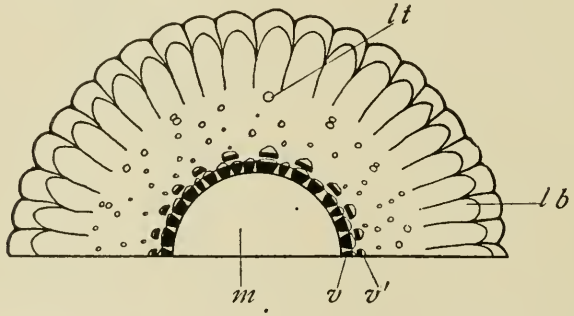


FIG. 3.— *Cycas media:* showing gross topography of transverse section of stem near tip; *v*, *v'*, 2 vascular cylinders; *m*, pith; *lt*, leaf traces; *lb*, leaf bases.

quite agrees with WORDSELL'S (*loc. cit.*) account of a situation exactly similar in *Macrozamia*. Fig. 1 represents the polyxylic structure diagrammatically, as it might be seen in radial section in the apical region of the stem. Differentiation which results in cortical cambium begins farther from the stem apex in each succeeding cylinder, being farthest in the outermost cylinder.

The cortex, true to cycadean character, is relatively large, as well as the pith. At the stem base the cortex measures 2.9 cm. between the outermost cortical cylinder and the leaf base, and at the tip 2 cm. A great many leaf traces traverse the cortex. At the base of the stem these traces are seen in longitudinal section (fig. 2), excepting at those places where they are just leaving the vascular cylinder. At the tip, however, leaf traces are invariably seen in transverse section (fig. 3), and they are characteristically double where they are about to enter a leaf base. Traces may leave any or all of the vascular cylinders, those from the inner ones passing to the cortex through the medullary rays of one or more outer cylinders.

DETAILED STRUCTURE

NORMAL CYLINDER.—Vascular bundles of the normal cylinder are long and narrow in transverse section (fig. 4), rarely becoming more than 3 or 4 cells in tangential thickness. Bundles taper to a rather sharp point toward the pith, and there is located the definite endarch protoxylem. WORDSELL had difficulty in locating protoxylem in the stem of *Macrozamia* which he studied, for it had been obliterated by the crushing caused by thickening wood cells. In the specimen which I studied the protoxylem is still intact in the majority of cases, and is easily distinguished (fig. 4). Fig. 5 represents protoxylem of the normal cylinder, enlarged enough to show its detailed character. The cell walls are less thickened than those of the primary xylem above, and there are certainly no pits present, as there would be if the xylem were of secondary origin. In this particular instance pits are absent from the primary xylem also. This is an unusual condition, since, as in fig. 6, primary xylem of the normal cylinder is practically always scalariform. Fig. 6 shows the radial aspect of the normal cylinder in its centripetal region. Here protoxylem elements are unquestionably spiral in character, while the succeeding primary xylem is scalariform. It should be said here that spiral tracheids are of comparatively rare occurrence even in the normal cylinder; at least they are rare in stretches large enough to be correctly interpreted. The usual form of protoxylem is scalariform rather

than spiral. Since neither transverse nor radial preparations show crushed masses of cellular material at the centripetal ends of bundles, there can be no doubt that the xylem elements which can be seen to terminate the bundles are truly protoxylem, whether they are spiral or scalariform.[8]

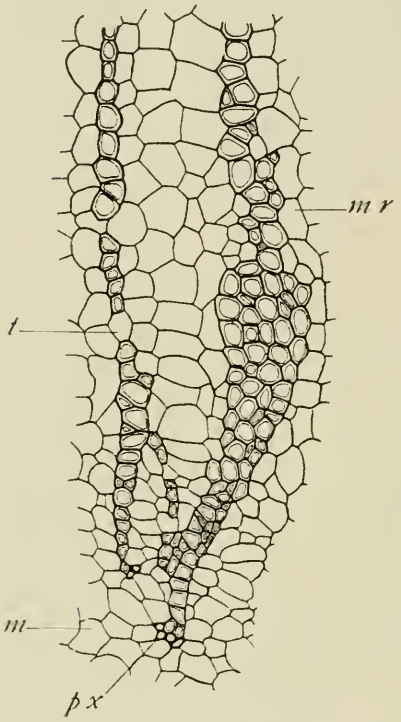


FIG. 4

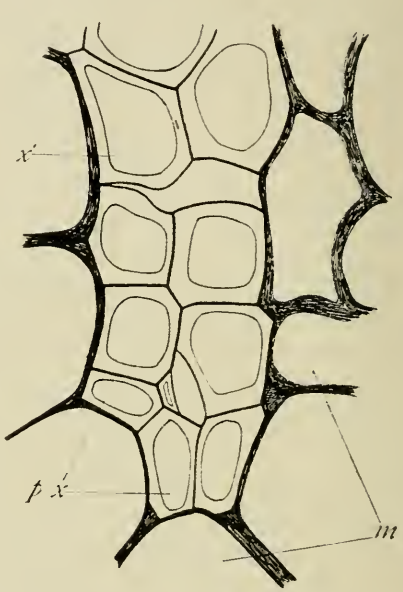


FIG. 5

FIGS. 4, 5.—*Cycas media:* fig. 4, transverse section of stem, showing only centripetal end of bundle of innermost cylinder; *m*, pith; *mr*, pith ray; *px*, distinct protoxylem; *t*, unthickened xylem elements; ×400; fig. 5, transverse section of stem, showing centripetal end of bundle of innermost cylinder highly magnified; *m*, pith; *px*, protoxylem; *x'*, primary xylem; pits apparent in none of these cells; ×850.

As in WORDSELL'S account, spiral tracheids here can be followed more easily in leaf traces off the normal cylinder than they can in the cylinder itself. The reason for this is not that these elements have been destroyed in the cylinder proper, but that

[8] CHAMBERLAIN, C. J., The adult cycad trunk. BOT. GAZ. 52:97. 1911.

they meander back and forth tangentially, so that only short patches can be caught here and there in a single radial section. The meandering habit is not so pronounced in the traces, and consequently longer stretches of primitive xylem elements can be seen and identified as such.

Secondary xylem of the normal cylinder is composed of tracheids which are characteristically pitted, the pits being confined largely to the radial walls, as described by both CHAMBERLAIN and JEFFREY.

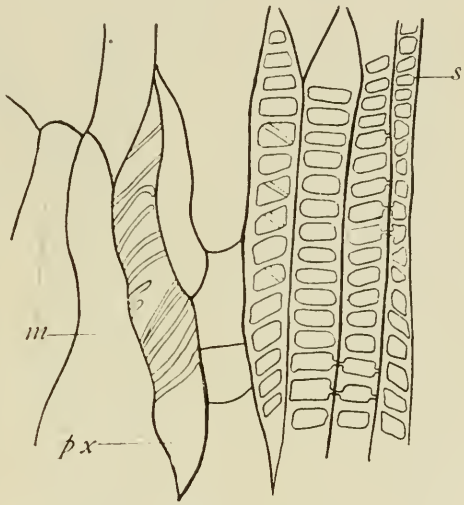


FIG. 6.—*Cycas media:* radial section of stem, showing centripetal end of bundle of innermost cylinder; *m*, pith; *px*, protoxylem distinctly spiral; *s*, scalariform tracheids of primary xylem, left one also having spiral thickenings; ×400.

The phloem situation of the normal cylinder adds emphasis to the fact of the latter's procambium origin, for protophloem is as distinct here as it is in any of the typical monoxylic cycad stems. Fig. 7 illustrates the upper phloem region of this cylinder, showing the crushed cellular substance which once was organized protophloem. This dark crushed mass has the appearance of a thick irregular ring in transverse section, entirely surrounding the normal cylinder and immediately inside the centripetal limits of the first cortical cylinder (fig. 8). The ring of course is interrupted here and there by medullary rays, but in many cases it extends unbroken across them, being squeezed in between the cells of the pith or cortical medulla. From this protophloem center primary and secondary phloem extend, fanlike, outward and downward in typical fashion. The rather startling character of the secondary phloem is its large number of suberized bast fibers compared to the number of sieve tubes. The former far

outnumber the latter, which occur here and there in short tangential rows between the bast.

FIRST CORTICAL CYLINDER.—The most intensive study of the cortical cylinder was made from preparations of the innermost one near the apex of the stem (fig. 3). Here details could be observed where the cylinder was in an early stage of development, and where its character could best be determined.

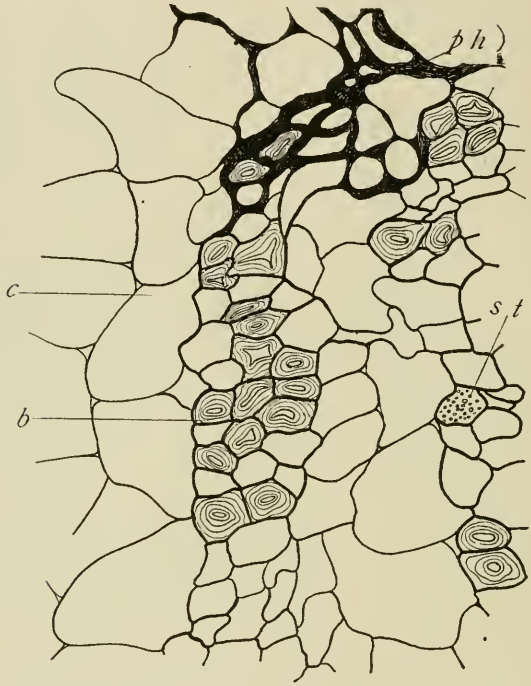


FIG. 7.—*Cycas media*: transverse section of stem, showing region of protophloem of innermost cylinder; *ph*, protophloem; *b*, suberized bast fibers of secondary phloem; *st*, sieve tube; *c*, cells of cortex; ×235.

Fig. 8 shows a transverse section of the entire cylinder, together with regions bordering it on both inner and outer boundaries. Apparently no protoxylem is present in these bundles. Cells at the centripetal limit of a bundle are not different from those nearer the cambium; there is little or no difference in size, shape, and thickness, in alignment, or in the character of cell

walls. Fig. 9 shows characteristics of the xylem in that region of the cylinder where protoxylem would be expected if there were any. All cells are uniformly thickened and, without exception, equipped with bordered pits, which is always a mark of secondary origin. In fig. 10 the same region is seen in radial section. Here the xylem element nearest the stem center, and even bordering on protophloem of the normal cylinder, is pitted. This one drawing illustrates tracheids of the first cortical cylinder which are as nearly scalariform as could be found; they are as rare as spiral tracheids are in the normal cylinder. By far the greater number of xylem elements in this centripetal region of the cylinder are pitted in exactly the same fashion as ordinary secondary tracheids of the normal cylinder, and they must in turn be considered as of secondary origin.

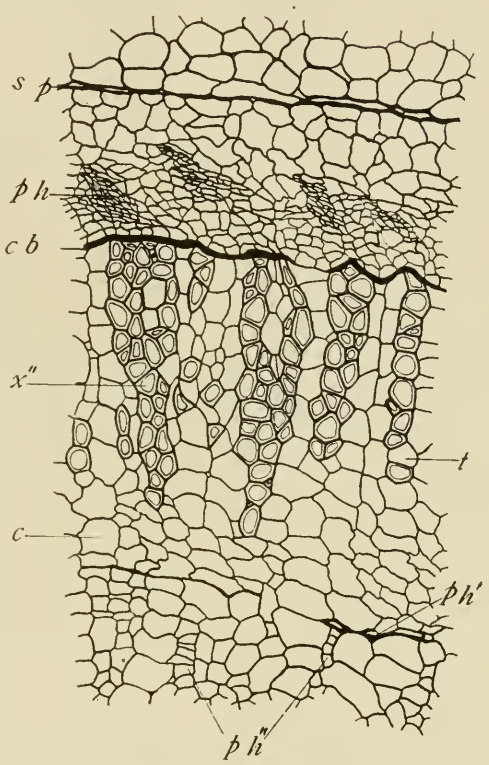


FIG. 8.—*Cycas media:* transverse section of stem, showing entire second cylinder as it appears near tip of stem; *ph'*, protophloem of first cylinder crushed; *ph''*, secondary phloem of first cylinder; *c*, cortical cells; *x''*, secondary xylem of second cylinder; *t*, unthickened xylem cells; *cb*, cambium; *ph*, secondary phloem of second cylinder; *sp*, split in cortex caused by expansion lower down of third cylinder; ×85.

In fig. 8 it will be seen that the region between the normal cylinder and the first cortical one is composed of purely cortical cells. Also the region between the first cortical cylinder and the split in the cortex, which marks the place where the second

cortical cylinder will appear, is purely cortical. No differentiation is evident between stelar pericycle and cortex; there is even no endodermis, and therefore there is no ground here for believing that the supernumerary cylinders originate in the pericycle. In view of Sister HELEN ANGELA'S[9] work with *Ceratozamia*, in which she found unquestionable cambiums at any place in the cortex from the stele to the periphery, it would be possible for the supernumerary cylinders of *Cycas* to originate in the cortex. In view of the evidence of fig. 8 it would also seem probable that the cylinders are truly cortical and not stelar.

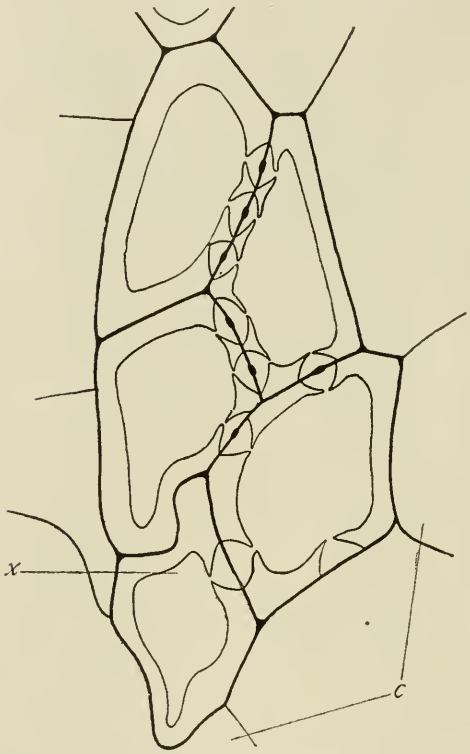


FIG. 9.—*Cycas media:* transverse section of stem, showing centripetal end of second cylinder; *c*, cortical cells; *x*, distinctly pitted xylem cells at tip of bundle; ×850.

There is no evidence of protophloem in connection with the cortical cylinder. A transverse section (fig. 11), which is thoroughly representative of the state of affairs, shows that practically all cells of the phloem are suberized bast fibers. This, together with the very apparent alignment of the fibers, is convincing proof that no protophloem is present.

Sections of the first cortical cylinder near the stem base reveal conditions almost identical with those of the normal cylinder, excepting that in the former both protoxylem and protophloem

[9] DORETY, HELEN A., The extrafascicular cambium of *Ceratozamia*. BOT. GAZ. 47:150–152. *pl.* 7. 1909.

are absent. Bundles of the former resemble those of the latter in shape, the secondary alignment of the former being disturbed by unequal growth and pressure, and bundles of both are of about equal size, those of the cortical cylinder being a little longer radially. Both cylinders give off leaf traces which differ in respect to the presence or absence of protoxylem and protophloem.

OTHER CORTICAL CYLINDERS.—There is little reason for believing that the second and succeeding cortical cylinders would have

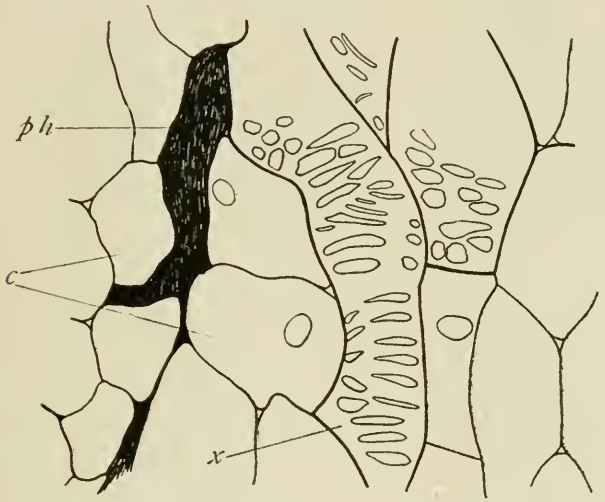


FIG. 10.—*Cycas media:* radial section of stem, showing centripetal end of second cylinder; *c*, cortical cells; *ph*, crushed protophloem of first cylinder; *x*, innermost xylem elements of cylinder, showing distinct pits, some having fused; ×400.

a mode of origin and development different from that of the first; consequently but little time was devoted to the study of the second cortical cylinder. Preparations from the stem base only were examined, and, as was expected, these showed conditions in the mature part of the stem identical with those of the first cortical cylinder in the same region. Further discussion, therefore, would be but a repetition of what has been recorded thus far.

In concluding the matter of cortical cylinders it may be well to mention the relationship of their number to the age of the plant. Certainly they do not occupy the position of growth rings, nor

are they laid down at regular intervals of time. The plant which was studied was more than a century old and yet had but 3 vascular cylinders. Doubtless the cortical cylinders are related to certain activities of the plant alternating with long periods of rest which may vary greatly in point of duration.

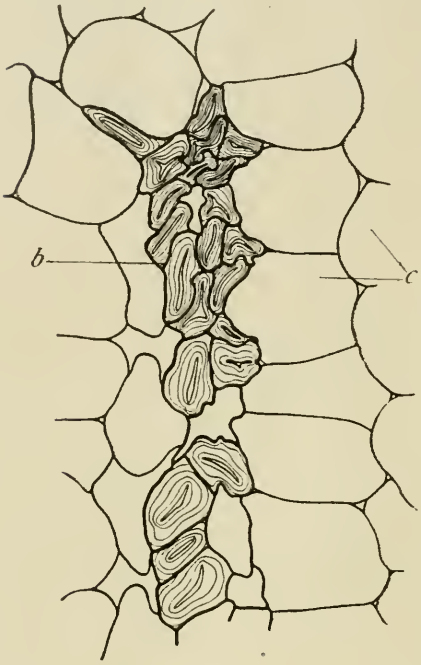


FIG. 11.—*Cycas media:* transverse section of stem, showing tip of phloem belonging to second cylinder; *c*, cortical cells; *b*, suberized bast fibers; ×235.

Summary and conclusions

1. The paper deals with the adult stem of *Cycas media*, particular attention being paid to the xylem and phloem details of the normal and first cortical cylinders.

2. Not all the vascular cylinders are of equal longitudinal extent. Only the normal one begins its differentiation as high up as the meristem, the others beginning their differentiation successively lower, and each one in the cortex outside the next inner cylinder. The normal cylinder, therefore, is the only one which would be expected to originate with a procambium, and the only one which could develop protoxylem and protophloem.

3. Following up these expectations, both protoxylem and protophloem were found to have been developed during the early activities of the normal cylinder. Protoxylem elements are usually scalariform, although hints of spiral tracheids are more or less frequent. Primary xylem is scalariform and secondary xylem is characteristically pitted.

4. Neither protoxylem nor protophloem was found in the first cortical cylinder. Practically all of the xylem elements are pitted, but scalariform tracheids are occasional.

5. The secondary phloem of both cylinders is characterized by the great number of suberized bast fibers compared to the number of sieve tubes.

6. All cortical cylinders are similar in respect to their origin and development and are probably related in their appearance to the alternating periods of rest and activity of the plant.

7. Unfortunately material was unavailable which would have shown the beginning of differentiation of a cortical cambium. Such a piece taken from the stem apex would have been far enough up to destroy material needed for further research.

University of Chicago

A PARASITE OF THE TREE FERN (CYATHEA)

F. L. Stevens and Nora Dalbey

(with plates xv, xvi)

Cyathea arborea (L.) J. E. Smith, one of the most beautiful of the Porto Rican tree ferns, is usually heavily infected by black fungous growths. Two collections of this fungus were made, one at Maricao, July 19, 1915, the other on El Alto de la Bandera, July 14, 1915.

On the older leaves the fungus is so abundant that the smallest frond segments, which measure about 3×7 mm., bear 25 or more of the black spots, and no segment is free of the fungus. On comparatively young fronds infections are less numerous, but even on such there are many fungous spots. A general idea of the appearance of the disease may be gained from figs. 1 and 2. The spots are often so abundant as to occupy considerably more than half of the leaf area. The individual spots are irregular in outline, and slightly elongated in a direction parallel with the veins of the host. The center of the spot is occupied by a conidiiferous structure, oblong, flattish, and dimidiate. This opens by an irregular crack, and in old pycnidia the whole top falls away (fig. 3). The cleavage lines seem to be determined by irregular rows of large cells. Immediately surrounding the pycnidium is seen a subiculum composed of close hyphae which appear to radiate much after the manner of the Microthyriaceae (fig. 4). Close focusing shows that this layer, instead of being superficial, is within the epidermal cells. Surrounding this epidermal subiculum is an area in which mesophyll cells alone are diseased. The diseased cells are dark brown and are quite filled with the coarse dark mycelium, while the adjacent cells are normal. In the cases of very young diseased spots, consisting of only a few cells, the infection is entirely in the mesophyll (fig. 19). It is only later that the central epidermal cells of a spot become invaded.

In microtome section these facts are verified: the mesophyll cells are seen to be invaded first, later the epidermal cells. Then the

fungus emerges and lays down a membrane one cell thick, of dark, thick-walled, closely woven hyphae. Under this arise in a close group numerous erect cells which in section appear like a palisade formation (fig. 6). These cells elongate; the covering layer becomes arched and eventually ruptures. The palisade-like cells are really conidiophores and bear the large, dark, 1-celled spores (fig. 8). The pycnidia are often sterile, the conidiophores then becoming greatly overgrown and distorted. The conidia germinate upon the surface of the leaf, producing at once an appressorium (fig. 9), which doubtless lends itself to the leaf invasion. This conidial structure is clearly of the Leptostromataceae-Phaeosporae, but does not fit well any of the form genera there given.

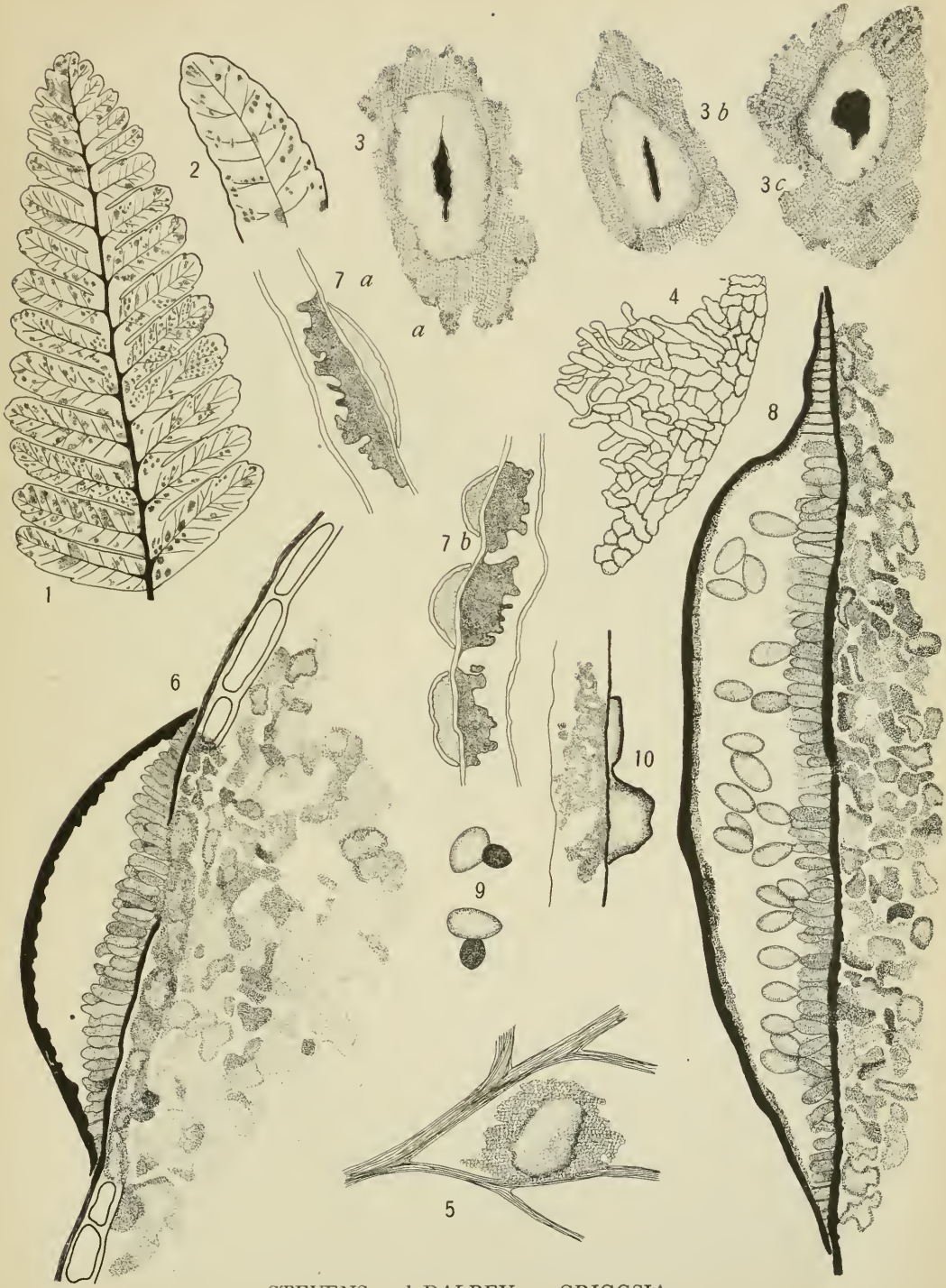
The infected spots give rise also to perithecia, although these are much less abundant. The perithecia at maturity are high and rounded (figs. 11*a*, 11*b*, 14, 15). The perithecial wall is composed of several layers of dark cells, compressed to a pseudoparenchyma (fig. 12). The perithecia are uniform in shape, with a domed top; that they arise from the same mycelium which produces the pycnidia is clear (fig. 10). The young perithecia are indistinguishable from pycnidia, and indeed it appears that a pycnidium which is not yet sporiferous can develop into a perithecium. The first indication of differentiation is that in the perithecia a bed of closely packed hyaline mycelium develops between the cuticle and the covering. Soon the top begins to arch and to lay on internally added layers in thickness. The pycnidium covering is only one cell thick, the perithecium covering always several cells thick. At maturity the perithecial wall is lined by a layer several strands thick of felted hyaline mycelial threads. The asci, which are not numerous, arise basally, various ages side by side, and interspersed with numerous long mycelial threads (fig. 14) which may be regarded as paraphyses, although they are far from typical paraphyses in appearance. The basal structure of both the pycnidia and the perithecia consists of a dense mat of mycelium laid down in the epidermal cells. This structure is difficult to represent because in the growth process the epidermal cells are largely obliterated. The facts, however, are hinted at in figs. 6, 12, and 15. It is this epidermal subiculum which gives the radiating effect shown in fig. 18.

The questions of morphology and parasitism of this fungus are of especial interest. The morphological characters to be emphasized are the internal intracellular mycelium, the external fungous layer which becomes the cover of a dimidiate pycnidium, the dome-shaped, flat-bottomed perithecium with a wall several cells thick and without ostiole. The habit of the mycelium of completely filling certain cells or irregular groups of cells while intervening and adjacent cells are entirely free of mycelium is striking. The whole picture gives a group of characters difficult to place satisfactorily. The mycelial characters are Microthyriaceous, the absence of ostiole Perisporiaceous, and it might be possible to regard the perithecial cavity as being in a stroma and thus incline toward the Dothidiaceae. It is also possible, when the top is fallen out of the perithecium, to regard it as Phacidiaceous, and it is here that we would place it, although the mode of formation and of opening of the perithecium are not fully characteristic of that family. The genus *Rhagadolobium*, described on a tropical fern (HENNING and LINDAU in Engler's Jahrb. **23**:288. 1896), presents certain similarities in the structure of the stroma, although it differs essentially in many ways, particularly in having the mycelium intracellular rather than intercellular, and in having 1-celled spores. In the Dothidiales the fungus resembles *Rhipidocarpon* Th. and Syd. in structure of the perithecium as seen in section, but the perithecium is not radial. In fact, it presents essential differences from all of the families of the Dothidiales as set up by THEISSEN and SYDOW. The fungus clearly shows differences from established genera sufficient to render its admission to any of them impossible. We therefore propose for it the new genus:

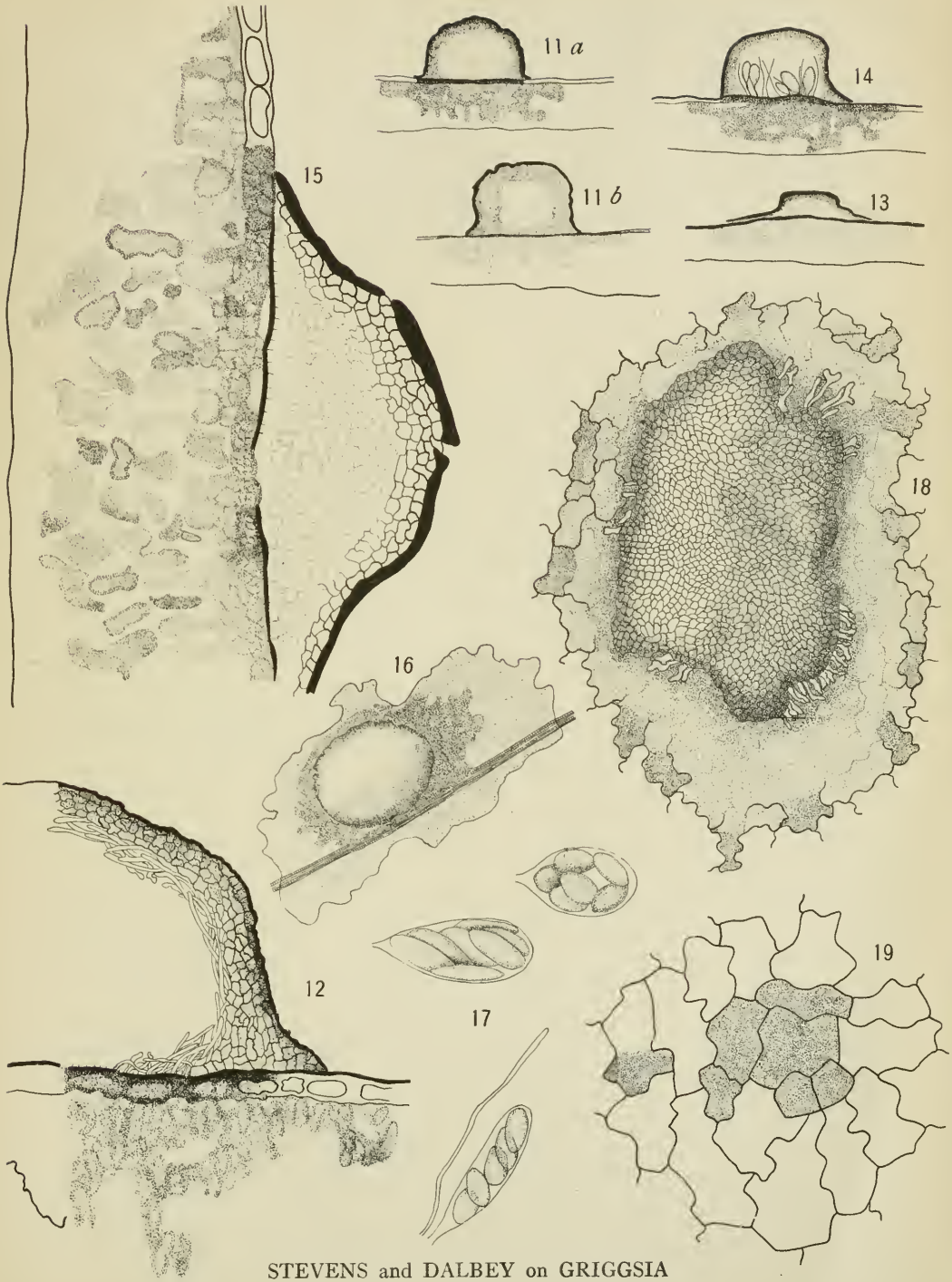
Griggsia, gen. nov.

Perithecia solitary, dimidiate, without ostiole, opening by irregular cleavage of the top, arising from a thin superficial and epidermal stroma, vegetative mycelium internal. Perithecial wall several cells thick. Asci basal, 8-spored. Spores oval, hyaline, 1-celled. Paraphyses hyaline, long, filamentous. Conidia in dimidiate pycnidia. Type species *Griggsia cyathea*. Named in honor of ROBT. F. GRIGGS.

Griggsia cyathea, sp. nov.—Perithecium dome-shaped, 200–300 μ in diameter, 150–160 μ high; wall about 24 μ thick on sides



STEVENS and DALBEY on GRIGGSIA



STEVENS and DALBEY on GRIGGSIA

and top. Asci 51×17–24 μ, ovate. Spores 10×17 μ, oval, hyaline, 1-celled.

Conidial stage: Pycnidia dimidiate, opening by a ragged cleft, amphigenous but more frequently epiphyllous, circular, 200–315 μ in diameter, 10–30 μ high, black, dimidiate, closely reticulate, reticulations about 7 μ in diameter, occupying the approximate center of a diseased spot. Spots irregular in outline, 1–3 mm. in diameter, very numerous, spores 1-celled, oval or pyriform, dark, obtuse, 28–34×14 μ.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

EXPLANATION OF PLATES XV, XVI

PLATE XV

FIG. 1.—Pinnule showing distribution of disease spots.

FIG. 2.—Frond segment with spots.

FIG. 3.—Top views of pycnidia with openings.

FIG. 4.—Detail of dermal subiculum showing radiation of fibers.

FIG. 5.—Drawing showing diseased mesophyll cells surrounding pycnidium.

FIG. 6.—Young pycnidium before spores are formed, showing covering layer and conidiophores.

FIG. 7.—Pycnidia showing shape and relation to host: shading indicates diseased cells.

FIG. 8.—Detail of pycnidium with spores.

FIG. 9.—Conidia germinating, with appressoria.

FIG. 10.—Pycnidium and perithecium produced from same mycelium: shading indicates diseased region.

PLATE XVI

FIGS. 11*a*–11*b*.—Perithecia showing typical dome shape.

FIG. 12.—Detail of perithecial wall.

FIG. 13.—Perithecium in early development with merely a bed of hyaline mycelium below the covering.

FIG. 14.—Perithecium showing asci and paraphyses; diagrammatic.

FIG. 15.—Young perithecium showing progressive thickening of cells and basal structure.

FIG. 16.—Pycnidium resting on dermal subiculum: second shaded area represents diseased mesophyll cells.

FIG. 17.—Asci and spores.

FIG. 18.—Top view of covering wall of pycnidium and of subiculum showing radiate character.

FIG. 19.—Very young infected area: only mesophyll cells diseased.

ANATOMY OF LYCOPODIUM REFLEXUM[1]

J. Ben Hill

(WITH FIVE FIGURES)

In a former paper,[2] giving in some detail the results of an investigation of the development and specialization of the steles of 6 species of *Lycopodium*, I reviewed the literature of the subject of the anatomy of *Lycopodium*. The anatomical studies have emphasized "types" of steles as characteristic of various species of *Lycopodium*, among which the radial and parallel-banded arrangements predominate. In my former paper I suggested that it was inadvisable to regard any stelar arrangement as characteristic of a species of *Lycopodium*, since almost all "types" may be found in a single species, and even in a single plant at different levels in the stem.

The plants of *L. reflexum* used in this investigation were collected by Dr. C. R. Barnes and Dr. W. J. G. Land in the vicinity of Xalapa, Mexico, in 1908. The habitat is described as moist clay soil. The material was preserved in a formaldehyde-alcohol solution and was given to me in this condition by Dr. Land, to whom I wish to express my thanks. The slides for the investigation were prepared from paraffin serial sections cut transversely 10–15 μ in thickness and stained in safranin–light green and in iron-alum haematoxylin-safranin, both combinations producing excellent results; the former is slightly better for differentiating protoxylem in sections of young stems, and the latter better for older tissues.

Investigation

In a study of the sections of the stem the most important matter of interest is concerned with the so-called "types" of stele to be found in *L. reflexum;* a secondary significant feature is

[1] Contributions from the Department of Botany of the Pennsylvania State College, no. 17.

[2] Hill, J. Ben, The anatomy of six epiphytic species of *Lycopodium*. Bot. Gaz. 58:61–85. *figs. 28.* 1914.

the presence of cortical roots. These originate in a manner similar to that described by Miss STOKEY[3] for *L. pithyoides*, and show a

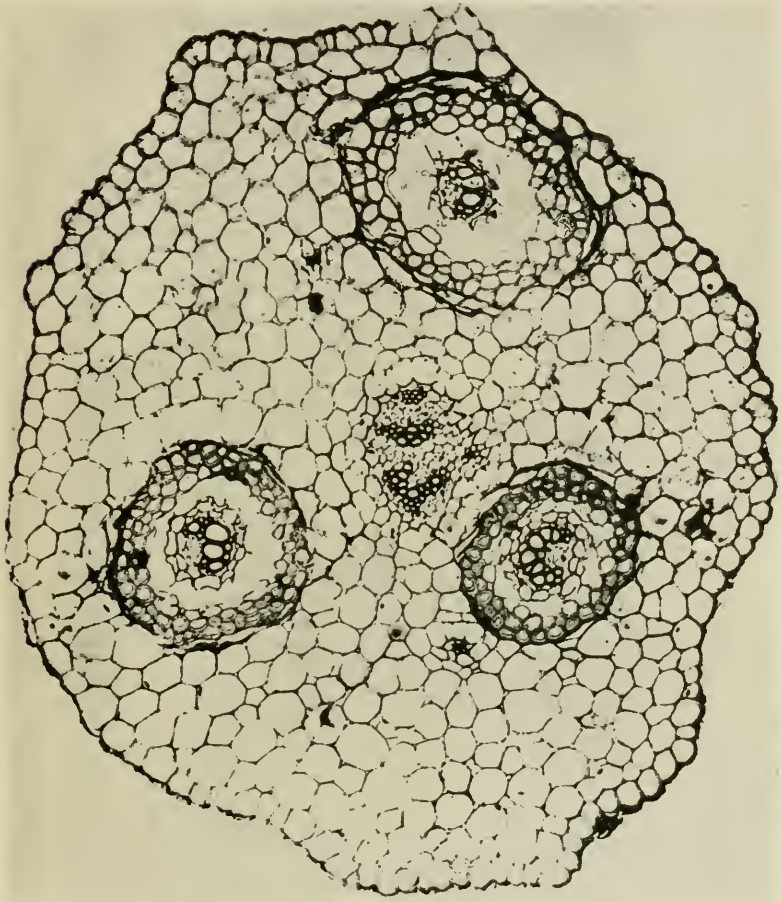


FIG. 1.—*L. reflexum:* transverse section of stem showing small stele and 3 cortical roots; in the roots phloem is surrounded by a crescent-shaped mass of xylem; root stele is surrounded by very thin-walled cells which generally are crushed and broken in sectioning; outer cortex of root composed of thick-walled cells; ×120.

development in the matter of differentiation of the stele paralleling that described[4] for the stem of *Lycopodium*, in which the xylem

[3] STOKEY, ALMA G., The roots of *Lycopodium pithyoides*. BOT. GAZ. 44:57–63. *pls. 5, 6.* 1907.

[4] HILL, J. BEN, *loc. cit.*

regions are recognizable long before lignification occurs. In young sections of the roots the metaxylem cells are recognizable early by their increased size and lack of protoplasmic content. The xylem is crescent-shaped, the protoxylem occurring at the ends and bordering the larger arc of the crescent. The mature roots, 2 or

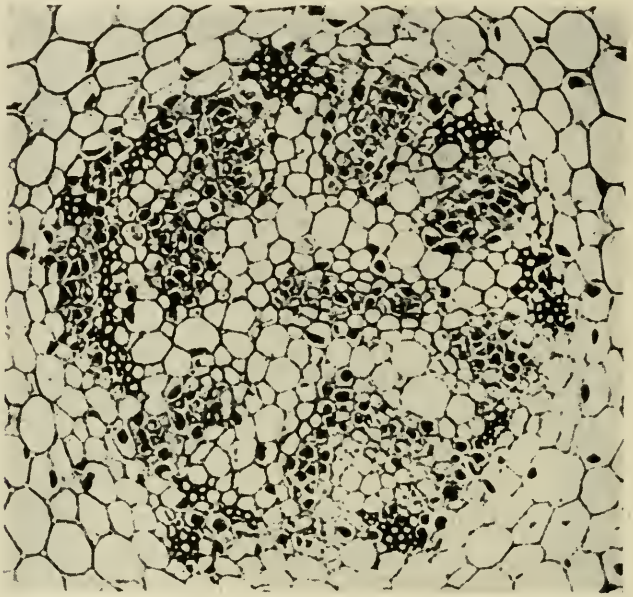


FIG. 2.—*L. reflexum:* transverse section through young stem tip showing several protoxylem points alternating with phloem in stele; phloem extends toward center of cylinder, alternating with unlignified metaxylem cells; there is a phloem island entirely surrounded by unlignified metaxylem cells; this arrangement when mature is represented in fig. 5; ×200.

3 in a stem, are typical cortical roots (fig. 1). The stem of *Lycopodium reflexum* is small, about 1 mm. or less in diameter, with a very small stele, 0.2 mm. in diameter.

In stating the results of the investigation of the steles of the stem of *L. reflexum* I shall include simply a brief description of the steles found in the stems of various ages, omitting the details of the development of the types, which were given in the former article and do not seem to vary much in different species.

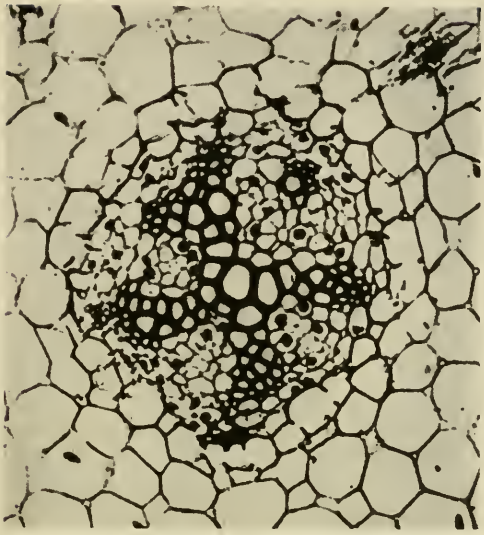


FIG. 3.—*L. reflexum:* stele showing radial arrangement of xylem with phloem located between protoxylem points and extending toward center of cylinder; ×200.

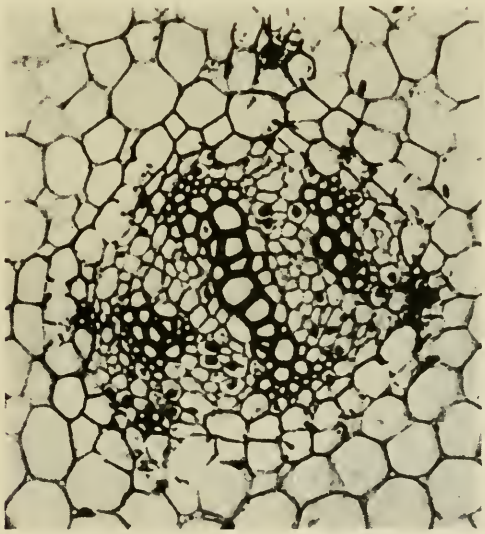


FIG. 4.—*L. reflexum:* stele showing parallel-banded arrangement of xylem with phloem occurring in bands alternating with xylem and extending across cylinder; ×200.

The number of protoxylem points in the stem stele ranges from 4 to 11, with about 7 as the most frequent number. Sections of the young stem tip show the condition characteristic of *Lycopodium*, in which at about the time of differentiation of the protoxylem points the metaxylem cells are distinguishable by their large size and lack of protoplasmic content (fig. 2). There are 3 so-called types of stele to be found in *L. reflexum:* the radial arrangement (fig. 3), the parallel-banded arrangement (fig. 4),

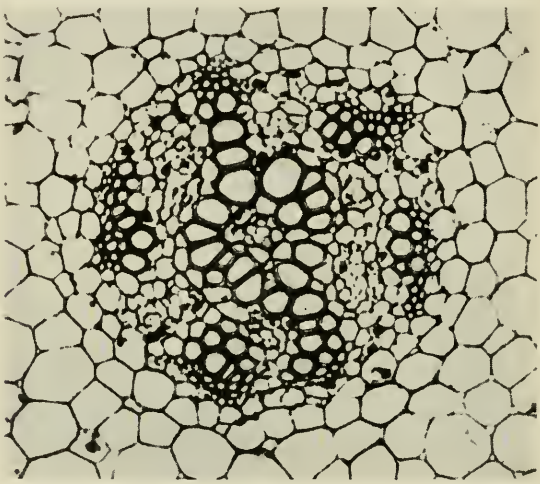


FIG. 5.—*L. reflexum:* stele showing inner cylinder of xylem surrounding strand of phloem, most frequent arrangement found in stele of *L. reflexum;* ×200.

and an arrangement consisting of an inner cylinder of xylem surrounding a strand of phloem (fig. 5). These 3 arrangements of the xylem may be found in the same stem at different levels, and are all modifications of the radial arrangement. The parallel-banded arrangement, in which alternating strands of xylem and phloem occur in parallel bands across the cylinder, seems to be correlated to some extent with the growth of roots, since this arrangement is to be found most frequently in the region where the roots arise. The arrangement most frequently found is that consisting of an inner cylinder of xylem inclosing a strand of phloem (fig. 5). From this cylinder strands of xylem radiate to

the protoxylem points. The condition is very similar to the characteristic stele described for *L. Billardieri*. The inner cylinder does not remain intact through any great length of stem, but is frequently broken up and gives rise to a parallel-banded arrangement or reverts to the radial arrangement.

Summary

1. The 2 points of interest in the study of the anatomy of *Lycopodium reflexum* are the presence of typical cortical roots and the various "types" of stele in the stem.

2. The development and differentiation of the tissues in the steles of the cortical roots parallel those in the stele of the stem.

3. There are 3 arrangements of the xylem: radial, parallel-banded, and a radial arrangement so modified as to consist of an inner cylinder of xylem inclosing a small strand of phloem. The last is the most frequently found.

4. The study confirms my former suggestions that all arrangements of xylem may occur in the same stem in species of *Lycopodium*.

The Pennsylvania State College
State College, Pa.

CURRENT LITERATURE

NOTES FOR STUDENTS

Permeability.—Further evidence against CZAPEK's theory of protoplasmic permeability is offered by Miss WILLIAMS,[1] who finds that immersion of tissues of *Saxifraga umbrosa* in certain electrolytic solutions, aluminum, potassium, and barium chlorides, potassium and barium nitrates, produces abnormal permeability to 0.2 per cent ferric chloride. Penetration was evidenced by reaction with tannin. The time of immersion required to bring about the change varied somewhat with the electrolyte, and greatly with the concentration employed. In all the electrolytes used, except barium chloride, plotting logs of time against logs of concentration in gram-mols per liter gave approximately straight lines. The abnormal permeability to iron chloride was induced without rendering the protoplasm permeable to colored cell sap in certain cells, and at surface tensions far from that considered critical by CZAPEK.—C. A. SHULL.

Colorimeter and indicator method.—DUGGAR[2] and DODGE have devised a method of obviating the interference of colored biological fluids with the indicator method of P_H determination. This is accomplished by placing equal layers of the colored test fluid on each side of a colorimeter; that on the left side receives the indicator, that on the right serves simply as a color blank. Equal layers of standard solution are also placed on each side; that on the right receives the indicator, that on the left serves as a blank.—J. J. WILLAMAN.

Flora of the Congo.—WILDEMAN[3] has resumed the publication of his studies of the Congo flora, which is prolific in new species. In the 5 fascicles just distributed, 334 new species are described, representing 39 families. The most largely represented families are Orchidaceae with 58 new species (27 of which belong to *Angraecum*) and Gramineae with 33 new species. Only 2 new genera are described, one in Leguminosae (*Pynaertiodendron*), and the other in Cucurbitaceae (*Bambekea*).—J. M. C.

[1] WILLIAMS, MAUD, The influence of immersion in certain electrolytic solutions upon permeability of plant cells. Ann. Botany 32:591–599. 1918.

[2] DUGGAR, B. M., and DODGE, C. W., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. 6:61–70. 1919.

[3] WILDEMAN, E. DE, Florae Congolensis. Bull. Jard. Botanique Bruxelles 4:361–429. 1914; 5:1–108. 1915; 5:109–268. 1916; 6:1–129. *pls. 35*. 1919.

A Laboratory Manual for Elementary Zoölogy

By LIBBIE H. HYMAN, Ph.D.

The Department of Zoölogy, University of Chicago

220 pages, 12mo, cloth; $1.50 (postpaid $1.65)

It is the purpose of this manual to include material that will introduce the student to the subject of zoölogy. Therefore it includes sections and appropriate exercises on general physiology, cytology, histology, embryology, heredity, classification, and ecology, although it is devoted in the main to a study of the structure of representative animals. In this way it is hoped that the student will gain a clear idea of the meaning and subject-matter of each of these branches of biology and their relation to the general subject.

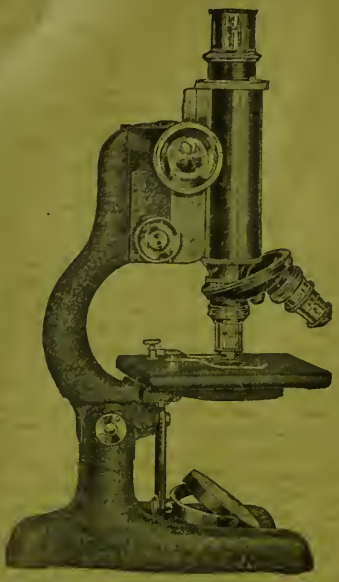
The manual begins with an exhaustive study of the biology of the frog, from which the student learns how a vertebrate animal is constructed, the function of each of its parts, and how these parts have come into existence. With the knowledge thus gained he is in a position to study other animals, which are taken up in their phylogenetic order, beginning with the Protozoa, and including representatives of the principal invertebrate phyla. In each case emphasis is laid upon the comparison of the animal at hand with the frog. By means of this comparison the student is led to appreciate the simplicity of the organisms, the order in which the functional systems of animals have arisen, and the way in which these systems become specialized with increasing complexity of structure.

Directions throughout the manual are detailed and precise, thus enabling the student to work by himself to the maximum degree. The purpose of each exercise and its bearing upon the general subject are given, all terms are defined, and the purpose and function of structures explained whenever possible.

The matter of which the manual is composed was previously used for three years in the Zöology Department of the University of Chicago and has met with the approval of all who have seen it. The feature which has been particularly commended is that each exercise has been described and explained in such a detailed manner that much explanation and labor have been spared on the part of both instructor and student.

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO - - - - - ILLINOIS



FS2 Model with two objectives in revolving nosepiece and one in eyepiece $53.25

Bausch and Lomb Microscopes

are again in the market—backed by the prestige which attaches to successful war service in any significant capacity.

Our release from this service is of course gradual. As the demands made upon us by the government are lessened from month to month, however, we are enabled to increase deliveries to our other patrons.

Write for complete catalog of our revised microscope line and inform us regarding your requirements.

Bausch & Lomb Optical Co.
412 ST. PAUL STREET, ROCHESTER, N.Y.
New York Washington Chicago San Francisco

Leading American Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

FINE INKS AND ADHESIVES
For those who KNOW

Higgins'

- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

At Dealers Generally

CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street Brooklyn, N.Y.

It Gives the Typist More Time to Type

These three things—*inserting the paper, typing, line spacing,* are all the typist has to do when she operates the

SELF-STARTING REMINGTON

Hand settings of the carriage, which are necessary on all other typewriters, are eliminated absolutely—and that means a time saving of 15 to 25 per cent in transcribing business letters. The Remington Self-Starter, or automatic indenter, gives the typist more time to type. That means a bigger day's work with no more effort; bigger typewriter value at no greater price.

REMINGTON TYPEWRITER COMPANY
(Incorporated)
374 Broadway New York

Volume LXVIII Number 4

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

OCTOBER 1919

Origin and Development of the Pycnidium - - - F. E. Kempton 233
(With Plates XVII–XXII)

Ecology of Tilia americana. I. Comparative Studies of the Foliar Transpiring Power. Contributions from the Hull Botanical Laboratory 253 - - - - - - - - - - James E. Cribbs 262
(With thirteen figures)

Repeated Zoospore Emergence in Dictyuchus - - William H. Weston 287
(With Plate XXIII and one figure)

Relation of Nutrient Solution to Composition and Reaction of Cell Sap of Barley - - - - - - - - - - D. R. Hoagland 297

Briefer Articles

Paraffin Solvents in Histological Work - - - - Paul Weatherwax 305

Current Literature

Book Reviews - - - - - - - - - - - - 307

Notes for Students - - - - - - - - - - - 308

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London and Edinburgh*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Volume LXVIII Number 4

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued October 16, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, £1 12*s.* 6*d.* each; single copies, including postage, 3*s.* 6*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 18 Peking Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

VOLUME LXVIII NUMBER 4

THE
BOTANICAL GAZETTE

OCTOBER 1919

ORIGIN AND DEVELOPMENT OF THE PYCNIDIUM

F. E. KEMPTON

(WITH PLATES XVII–XXII)

Introduction

The Ascomycetes have been the subject of repeated investigations, especially in regard to the morphology of the perithecium and the cytology and sexuality of the ascigerous stage. The conidial stages, placed in the Fungi Imperfecti in the absence of knowledge of their ascigerous stages, of necessity are classified on a basis of the morphology of the conidia-bearing structures. For this reason, and because the pycnidia are said sometimes to develop directly into perithecia, extension of our knowledge of the morphology of such conidiiferous structures as the pycnidium and the acervulus is highly desirable.

The references in literature to the development of the pycnidium are, for the most part, merely incidental, and studies directly devoted to the subject are few. As early as 1876 BAUKE (5) described what he considered as 3 methods of pycnidial development, without giving names to them. In 1884, however, DEBARY (9) distinguished 2 main methods of development, which he designated as "symphogenous" and "meristogenous": "symphogenous" when the pycnidial primordium arises through the interweaving of young hyphal branches to form a network that is at first loosely woven, but later becomes compact and knotlike;

"meristogenous" when the primordium arises from a single hyphal cell or a group of adjacent cells of a single hypha by continued cross and longitudinal division. In the latter, branches from neighboring cells or from the mass itself may share. A number of variations of these 2 methods have been described. Classification of the pycnidial developments described by BAUKE according to the methods distinguished by DEBARY places *Cucurbitaria* as meristogenous, *Diplodia* as symphogenous, and *Pleospora polytricha* as a combination of the two.

ZOPF (52) in 1890 mentioned 3 types of pycnidial development: "Hyphenfrucht," "Gewebefrucht," and "Knäuelfrucht." The "Hyphenfrucht" comprises those in which development proceeds from a single hyphal cell which by dividing and swelling forms a quadrant, from which with the aid of 2 or 3 neighboring cells a primordium is formed. By "Gewebefrucht" is designated those instances in which a tissue mass is formed. This mass may develop in either of 2 ways: by neighboring cells of a hypha becoming septate, swelling, dividing, becoming more rounded, while short dividing hyphae either from the mass or from neighboring cells of the hypha share in the formation; or in a like manner it may be formed from cells of 2 or more contiguous hyphae. The "Knäuelfrucht" develops a primordium from 1 or more short hyphal branches which coil spirally, branch, and interweave into a knot which later develops into the pycnidium. The "Hyphenfrucht" and "Gewebefrucht" are clearly variations of the meristogenous method of development of DEBARY. The other, "Knäuelfrucht," is symphogenous.

In addition to these should be mentioned the "sporopycnidium" of VON TAVEL (46), referred to also by PLANCHON (29) and SCHNEGG (33). This pycnidium arises meristogenously, according to VON TAVEL, representing the most extreme case of meristogenous development. A single spore in the presence of abundant food germinates and grows a short mycelium; then by division and growth becomes a pycnidium. REDDICK (31) notes that pycnosclerotia may be found in *Guignardia*. These bodies arise as pycnidia, produce pycnidiospores, and later function as perithecia, producing asci and ascospores.

Although in many instances the fungi were not studied primarily as to their pycnidial development, and such information as was collected regarding this was incidental, according to the figures and descriptions given the following belong to the meristogenous group of DeBary: *Pleospora* (Gibelli and Griffini 17; Bauke 5), *Cucurbitaria* (Eidam 14; Bauke 5), *Pycnis* (Brefeld 6), *Fumago* (Tulasne 43; Zopf 53; Schostakowitsch 34), *Sphaeronaema* (Halsted and Fairchild 18), pycnidium with *Teichospora* (Nichols 27), *Phyllosticta* with *Alternaria* (Planchon 29), *Sphaeropsis* and *Coniothyrium* (Potebnia 30), *Chaetophoma* (Arnaud 3), *Phoma* (Reddick 31; Mercer 26; Schnegg 33), *Endothia* (Anderson 1). As symphogenous may be classed: *Cicinnobolus* (DeBary 8), *Fumago* (Tulasne 43; Zopf 52); *Diplodia* (Bauke 5; Van der Bijl 44), *Graphiola* (Fischer 15), *Cystispora* (von Tavel 46), *Hendersonia* (Voges 45; Wolf 51), pycnidium with *Sphaerella* (Higgins 21), *Sphaeropsis* (Hesler 19), *Septoria* (Levin 25). A few of the species have both types of development.

The present investigation is an endeavor to extend our knowledge of the very early stages in the development of the pycnidium and kindred structures.

Methods

For the present study, the fungi were either isolated from their hosts or cultures were procured from various sources as noted. Corn meal agar (35) was used because it gave an abundant growth, and its transparency facilitated the study of even the youngest stages. Other media were used in a few instances for comparison. Cultures for study were grown in Petri dishes, at room temperature (15–37° C.). Transfers were made by lifting hyphae or spores from pure cultures to the poured agar in Petri dishes. Drop cultures of melted agar were made by the use of sterile pipettes. In some cases the drop of agar was inoculated with spores as for dilution plating. In others, each drop was inoculated by transfer. These drop cultures served well for study of early stages of development, but seldom produced mature conidiiferous structures.

Disks of agar containing young pycnidia were removed from Petri dish cultures, placed on cover glasses, and inverted on

Van Tieghem rings in order to follow further the development of the pycnidia. Growth in Petri dishes was observed periodically, the intervals depending upon the rate of growth of the organism. Pycnidia of each species were kept under observation during their entire period of early development. For close detailed study and for drawing, mounts were made by removing from Petri dish cultures small squares of agar containing pycnidia in various stages of development, mounting them either in water for immediate study, or in glycerine for later study. Material from test tube cultures was teased out and studied for comparison and verification of many points.

Following the usage of earlier writers, DEBARY (9), BAUKE (5), ZOPF (52), and VON TAVEL (46), the word primordium is used throughout this paper to designate a group of cells that have become so differentiated that it is clearly evident that from them a pycnidial or similar structure will arise.

Genera and species studied

PHOMA (Fries) Desmazières

Phoma herbarum West; isolated from its host, *Polygonum hydropiper* L., at Urbana, Illinois, July 8, 1916.

Pycnidia are produced in cultures in moderate abundance. All stages of development, from the mature pycnidia on older mycelium to beginning stages on the younger mycelium, can be observed even in a small sector of a Petri dish culture. Development proceeds typically from a single cell of a hypha. This cell divides both transversely and diagonally (fig. 1) into a few cells which swell and enlarge. These cells by continued swelling and dividing form an irregular mass with very few or no hyphal branches. This mass shows distinctly its origin from a single hypha (figs. 1–3). As the mass continues to enlarge, one side protrudes slightly as a short rostrum which becomes lighter in color (fig. 4). This is the primordium which later becomes a pear-shaped pycnidium with an ostiole from which spores are discharged (fig. 5). The development is a typically simple meristogenous one.

Phoma pirina (Fries) Cooke; isolated at Urbana, Illinois, May 10, 1916, from dead twigs of *Pyrus communis* L. from Savoy, Illinois.

This fungus produces abundant small pycnidia 60–120 μ in diameter, at first closed but later with an ostiole 20–30 μ wide. The spores vary in size from 5×3 to 10×4 μ. A few cells of the hypha swell, divide both crosswise and diagonally, swell, and divide again (figs. 6, 7). This small mass continues to enlarge by cell division, and a few hyphal branches bud out from it (fig. 8). By continued development an irregular mass (fig. 9) is formed, which is the primordium of the pycnidium. In a few cases closely lying hyphae may take part in the formation (figs. 10–13), but the typical method of development is the simple meristogenous one from a few cells of a single hypha.

Phoma destructiva Plowr.; isolated at Urbana from fruits of tomato (*Lycopersicon esculentum* Mill.) in September 1915. In all respects the disease and the fungus agreed with the one described by Miss JAMIESON (22).

The pycnidial primordium usually arises meristogenously. In most cases a single hypha gives rise to the primordium (figs. 14–17); in other cases 2 or more hyphae share in its formation (figs. 18, 19, 22). In either case adjacent cells swell and divide crosswise and diagonally until a rounded or elongated mass is formed, slightly darker in color than the mycelium. The former (fig. 15) is the simple type of meristogenous development.

Phoma, species indet.; isolated from stems of clover (*Trifolium pratense* L.) at Urbana, Illinois, in July 1916.

Development takes place in a simple meristogenous manner. One hypha only is involved in the formation of the primordium (figs. 23–27). Short hyphal threads sometimes branch from the mass. The cells are usually large and swell into a rounded shape. The mass develops more rapidly at one end than at the other, so that the young pycnidium is slightly conical.

Phoma, species indet.; isolated from a berry of the Concord variety of grape (*Vitis labrusca* L.), from a vineyard near Collinsville, Illinois, September 1917.

A portion of the culture seen under the high power of the microscope showed a very characteristic arrangement of hyphae and of

pycnidia in various stages of development (figs. 28–33). The simple meristogenous development of the primordium is similar to that described for *Phoma herbarum* West (figs. 1–5).

Phoma cichorii Passr.; isolated from *Phlox divaricata* L. by Mrs. ESTHER YOUNG TRUE, at Urbana, Illinois, November 1917.

A few adjacent cells of a single hypha or of a number of contiguous hyphal strands divide into short cells (figs. 34, 35). By budding and swelling, short hyphal branches arise, the cells of which swell and divide (fig. 36). The cells of the mother strand or strands also enlarge and divide. The component hyphae anastomose, forming a pseudoparenchymatous, irregularly rounded mass. This mass develops into the pycnidium. The development of this fungus differs from that described for the other species in that, where only a single strand is involved, the mode is simple meristogenous with the original cells and their budding branches anastomosing to form the mass (fig. 36). In instances where 2 or more strands are involved (fig. 37), there has been a similar division, swelling, and branching in each strand, but the whole has been united into a single primordium as described for *Phoma destructiva* (fig. 22). This mode is compound meristogenous.

MACROPHOMA (Sacc.) Berl. and Vogl.

Macrophoma citrulli (B. and C.) Berl. and Vogl.; isolated by Dr. J. A. ELLIOTT from a cantaloupe (*Cucumis melo* L.) leaf procured from Alabama in the autumn of 1915.

Young pycnidia and small sclerotia form in abundance, soon rendering the culture dark brown or black. Usually a single cell in a hypha becomes slightly swollen. It then divides into 2 short cells (fig. 38). These cells in turn divide by cross, longitudinal, and diagonal walls (figs. 39, 40). The swelling continues until the body becomes a rounded or oblong mass much darker than the light brown mycelium (figs. 46, 47). The cells immediately adjacent to the dividing mass may divide into short cells and send out hyphal branches (figs. 42–44). The outer cells of the mass may also send out branching hyphae. The hyphae from all these sources form a network about the enlarging mass. Sometimes the hyphal branches fuse with the mass, and in other cases they appear to

have no part in its formation. The body thus developed is the primordium from which the pycnidium arises. It continues to enlarge, becomes pear-shaped, forms an ostiole, and develops spores. This mode of development is meristogenous, with a slight modification in that short hyphal branches either fuse with or form an envelope about the primordium.

Less frequently a second method appears, in which short branches from main mycelial strands direct themselves toward a point. There these branches interweave, swell, and divide to form a network (fig. 48). This network is at other times formed by the looping back or snarling of hyphal branches. The tangle enlarges, becomes more tightly woven, then the cells divide, swell, and anastomose to form a pseudoparenchymatous mass (fig. 49). This method is symphogenous. This species gives rise to both meristogenous and symphogenous developments.

The pycnidia of the species studied in the genera *Phoma* and *Macrophoma* indicate that both meristogenous and symphogenous methods of development may be found. These species present in the main the simple meristogenous mode in which a few cells of a single hypha take part (figs. 1, 6, 15, 23, 29, 38, 44); also a slight variation of the simple development in which hyphal branches both from the dividing cells and from other cells of the mother hypha form a network about the original cells taking part in the development .(figs. 36, 39, 47). The compound meristogenous development is that in which 2 or more main hyphae, lying parallel or crossing, begin to divide into short cells at a point of contact, swelling and dividing to form a primordium (figs. 18, 22, 37). A few adjacent cells of each hypha and some surrounding branches take part in this type of development.

Sphaeronaema Fries

Sphaeronaema fimbriatum (E. and H.) Sacc.; procured from Dr. Byron D. Halsted, October 1916.

Halsted and Fairchild (18) report and figure the development of the pycnidium of *Ceratocystis fimbriata* as follows:

> In its initial stages the pycnidium arises as the swollen and curled or twisted tip of a vegetative hypha, or as a twist or knot in a sporophore between

the conidium and its point of union with the main hypha. Although observed to be present in numerous cases, no anastomosing of different hyphae branches seems necessary. Almost simultaneously with the first curving of the hypha tip side branches arise, which, by their growth and formation of septa, form the coarsely cellular membranous wall of the pycnidium.

Single mountings from plate cultures gave all stages of development verifying this description. The usual development was the twisting, budding, branching, and enlarging by cell division of single twisted hyphal branches (figs. 50–52). This is a variation of the meristogenous development.

Sphaeropsis Léveillé

Sphaeropsis malorum Pk.; isolated from apple fruits (*Pyrus malus* L.) from Centerville, Indiana, in October 1916.

Hesler (19) gives the development of the pycnidium of *Physalospora cydoniae* Arnaud in agar cultures as follows:

> The dense pseudoparenchyma of the maturer fruit bodies suggests meristematic divisions, but apparently the structure, for the most part, arises symphogenetically. In agar cultures a group of threads may be observed to be directed toward a common point where the pycnidium is to be formed. Here the hyphae are composed of cells 6–7 μ broad, their length varying from 20 to 70 μ, always longer than broad. In the region where the pycnidium is to be developed, the cells become noticeably shorter by the laying down of new walls; the cells also increase in diameter by growth, and the hyphae increase their numbers by branching. The interspaces found in the earlier stages are filled by the growing in of these branches and by a budding-like action of the hyphal cells bordering the space.

Among my own notes upon *Sphaeropsis malorum* Pk., made before the appearance of this description, is an account of the development of the primordium which it seems worth while to give here.

The pycnidial primordium of this species is formed usually according to the method designated by DeBary as symphogenous. A number of hyphal branches interweave near their ends (fig. 53); these in turn branch and the branches also interweave (figs. 54, 55). The central portion becomes a tightly woven mass. The whole continues to enlarge until a spherical mass is formed, which develops into a pycnidium. Most of these primordia form deeply

imbedded in the agar, although a few develop on or near the surface. Those developing imbedded in the agar are smooth, while those on the surface have a fuzzy appearance. A very few pycnidia form by the compound meristogenous method described for *Phoma*. These usually form 2 or more contiguous hyphae in which a few adjacent cells divide, swell, continue to divide, and enlarge until a primordium similar to the one described is formed.

Sphaeropsis citricola McAlpine; isolated at Urbana, Illinois, January 1916, from a kumquat fruit (*Fortunella margarita* Swingle) from Lake City, Florida. The condition produced on the rind of the fruit was that of a very black carbonaceous spotting which finally spread over the whole fruit.

In culture a coarse brown mycelium 5–8 μ in diameter is produced. Numerous pycnidia 80–175 μ in diameter develop from primordia each of which arises either from a single cell or a few adjacent cells in a single hypha. This cell or group of cells (figs. 56, 57) divides by cross and diagonal walls (figs. 58–61), later by the addition of longitudinal walls to form a rounded or elongated mass (figs. 62, 63) which is slightly darker in color than the mycelium. This mass becomes globose, light brown, carbonaceous and reticulated, and is the primordium. Development is usually simple meristogenous. The compound meristogenous mode is seldom found (figs. 64–66). From pycnidia developing from these masses small hyaline spores 5–7 $\times$ 4–5 μ exude.

The genus *Sphaeropsis* presents a variation of the symphogenous development in that the branches interweave near their ends at a point some distance from the main mycelial strands (fig. 53). It also shows the more usual symphogenous type (figs. 54, 55) described for *Macrophoma* (figs. 48, 49). Simple meristogenous development occurs in one species. The compound mode seldom occurs in the species studied.

Coniothyrium Corda

Coniothyrium pyriana (Sacc.) Shel.; pure culture isolated from twigs of apple (*Pyrus malus* L.) from Savoy, Illinois, October 1916.

A few adjoining cells of a main hypha become slightly swollen and divide into short cells (figs. 67, 68). These cells swell, divide

by cross, diagonal, and longitudinal divisions, and continue to swell and send out short hyphal branches by budding (figs. 69, 70). This body increases in size, becoming darker in color. Branches from adjoining cells or closely lying hyphae may interweave with the branches from the mass, at length forming a primordium (fig. 71). from which a pycnidium develops. Usually this primordium arises from a single main hypha, but occasionally 2 or more contiguous hyphae are included in its formation. The mode of development is meristogenous, with small hyphal branches at times included in the formation. Both simple and compound modes are found, but the compound mode seldom occurs.

Coniothyrium, species indet.; culture procured from the air of the laboratory in the summer of 1917 by W. S. BEACH.

A few adjacent cells in a single hypha divide into shorter cells. These cells branch profusely (fig. 72). A few of the cells continue to divide and swell, and the branching hyphae divide and branch (fig. 73). The whole becomes a more closely formed mass (fig. 74) which forms a globose, dark brown, finely reticulated primordium. From this the pycnidium develops. The mode of development is simple meristogenous.

The genus *Coniothyrium*, so far as studied, presents only the meristogenous development.

SEPTORIA Fries

Septoria polygonorum Desm.; isolated from its host *Polygonum persicaria* L. at Urbana, Illinois, July 1916.

The development of the primordium in most cases is by the usual meristogenous method, but in some instances it is atypical. Some of the primordia arise from a single hypha, 1 or 2 cells of which divide transversely and diagonally, and, swelling slightly, continue to divide (fig. 75). Later this mass develops into a globose, finely reticulated, pale brown body. In some instances the compound mode appears in which 2 or more hyphae are involved in the formation (fig. 76). In other cases primordia are to be found arising from a main hyphal strand with numerous branches of nearby hyphae intermingling in the mass (fig. 77). This involves both the meristogenous and the symphogenous

methods, and so may be considered a combination of the two. The principal method of development is meristogenous.

Septoria scrophulariae Pk.; isolated from its host *Scrophularia leperella* Bicknell by Mr. Walter S. Beach during the summer of 1917.

In pure culture it produces a closed sporing body, the primordium of which arises either from a few cells of a single hypha or from 2 or 3 closely lying hyphae (figs. 78, 79). These primordial cells divide, swell, and continue to divide to form a small ball-like structure which later develops into 1 or more pycnidia. They thus arise either by the simple or compound meristogenous mode.

Septoria helianthi E. and K.; isolated from its host *Helianthus grosseserratus* Mortens by Mr. Walter S. Beach in the summer of 1917 at Urbana, Illinois.

Pycnidia form readily in culture. A single cell or a few adjoining cells in a hypha become slightly swollen. These then divide into shorter cells which swell and send out very short branches (fig. 80). The swelling continues until a rounded mass is formed, slightly darker in color than the pale brown mycelium (fig. 81). The body thus formed becomes almost globose in shape. The outer portion or covering becomes membranous with a cellular appearance. This primordium continues to enlarge, and becomes ovate or elliptical in shape. The development is simple meristogenous, within a single strand of mycelium.

The genus *Septoria* can hardly be judged by these few species, but these, with those reported in the literature, indicate that the 2 main methods of development, namely, meristogenous and symphogenous, and even a combination of these two, may occur.

Sphaeronaemella Karst.

Sphaeronaemella fragariae Stevens and Peterson; procured from Dr. Alvah Peterson, Urbana, Illinois.

A few cells of a single hypha divide, producing very short cells. These bud and branch profusely, usually in one direction, into short hyphae (fig. 82). These branches anastomose (fig. 83), twining about in a circular manner. They then divide and swell, forming a small mass which usually protrudes from one side of the main

mycelial strand from which it arises (fig. 22). The body thus formed is the primordium from which the pycnidium arises.

In the genus *Sphaeronaemella* a striking variation of the meristogenous development is found. A few cells of a main mycelial thread begin to divide as in the simple meristogenous mode, but short hyphal branches arise from these, usually on one side, curling and twisting about each other to form a ball-like mass. In some cases this mass envelops and includes the main hypha (fig. 84).

GLOEOSPORIUM Desmazières and Montaigne

Gloeosporium rufomaculans (Berk.) Thüm.; isolated from fruit of apple (*Pyrus malus* L.) from Neoga, Illinois, September 1917.

The primordium of the acervulus arises from a number of neighboring hyphae. These branch into short hyphae which branch in turn, forming a loosely woven network. A spreading tuft arises from this loosely woven base (fig. 87). This cushion with its tuft of short hyphae is the primordium. It usually originates symphogenously, sometimes meristogenously.

Gloeosporium musarum C. and M.; isolated from a banana (*Musa sapientum* L.) from the Champaign market, July 28, 1916.

Short hyphal branches from main mycelial strands interweave near their ends. Other hyphae intertwine about this initial portion. Some branches fold or loop back upon themselves, while still others branch again. The cells of the interwoven mass divide, swell, and branch (fig. 88). At length a cushion-like base is formed. This is the primordium of the acervulus, from which short conidiophores arise which bear conidia. In terms of pycnidial development it arises symphogenously. In culture this fungus bears many spores, either sessile or upon short conidiophores outside of acervuli, scattered freely upon the mycelium. These spores appear before the formation of acervuli, making the study of the beginning of acervuli difficult.

COLLETOTRICHUM Corda

Colletotrichum lagenarium (Pers.) E. and H.; isolated from a watermelon (*Citrullus vulgaris* Schrad.) procured on the Champaign market, September 1917.

The primordia of the acervuli begin their formation deeply imbedded in the media, even near the bottom, by the time the culture is 4 cm. in diameter and no more than 4 or 5 days old. At this age no conidia have developed upon the short conidiophores or as buds from cells of hyphae, as often occurs when the culture becomes older. The primordia arise by 2 methods: (1) from a few cells of a main hypha arise a few or many short budlike hyphae (figs. 89–92); these elongate, intertwine, and branch to form a cushion-like base from which very short conidiophores arise; (2) hyphal branches from a few neighboring or contiguous mycelial strands intertwine, some of the threads forming short loops which mass, intertwine, and branch (figs. 93–96), forming an irregularly shaped, loosely made, cushion-like base from which conidiophores arise as in the other type. Irregular large acervuli, or acervuli-like groups, bearing numerous conidia, arise in this latter type. The first mentioned type may be considered as meristogenous, both simple and compound modes appearing; the second is symphogenous.

The genera *Gloeosporium* and *Colletotrichum* have been extensively studied by SHEAR (36), STONEMAN (42), SOUTHWORTH (38, 39), EDGERTON (11), and others. Late stages of development and cross-sections of different stages have been figured from fixed material in host tissues, but little has been said in regard to the early development of the acervulus. The 3 species studied give insight only into the origin of the cushion-like base from which the acervulus arises. They present 2 distinct types, the simple loosely woven base that arises from a single hypha or from a few contiguous hyphae, and the more complexly interwoven base which arises symphogenously.

PESTALOZZIA De Notaris

Pestalozzia palmarum Cke.; pure culture procured from the Centralstelle für Pilzkulturen, Amsterdam, Holland.

A few hyphae, usually 2–6, lying side by side form a bed in which a few cells begin to divide into shorter cells. These cells may be in only one of the hyphae (fig. 97*a*), or they may be contiguous cells of 2 or more hyphae (fig. 100). These cells swell and continue to divide by cross and longitudinal walls, at length forming

a protruding oval or obovate body (fig. 97*b*) which is the primordium of the pycnidium-like body which later forms. This primordium arises by either simple (fig. 97*a*) or compound meristogenous (fig. 100) development. It soon becomes globose and membranous, with a cellular outer wall, but remains light of color, so that a few dark spores may be seen within (figs. 98, 99). At this stage the structure is to all appearances a young pycnidium (fig. 101). Within a few days the top breaks, the few spores already formed are extruded, sometimes rather forcibly (fig. 102), and the cavity then becomes saucer-shaped. Conidiophores arise in profusion, and many spores are produced. When the first spores are noted the body is a pycnidium, if observed in a later stage it appears like an acervulus. In *Pestalozzia capiomanti* similar facts were noted by BAINIER and SARTORY (4), while LEININGER (24) in his studies of *P. palmarum* speaks of these bodies as pseudopycnidia.

Pestalozzia guepini Desm.; isolated from the fruit of a kumquat (*Fortunella margarita* Swingle) from Lake City, Florida, January 1916.

A few pycnidia were produced in plate culture. In the beginning stages a number of branches of closely lying or nearby hyphae branch, snarl, and intertwine (figs. 103, 104). Cells within the more closely twined part of the mass swell and divide by continued cross and longitudinal divisions until a rounded mass is formed, which is the primordium of the pycnidium (fig. 105). This primordium arises symphogenously. The development of the final sporing body from this stage is very similar to that described for *Pestalozzia palmarum*.

Pestalozzia, species indet.; isolated, by the author November 1916, from leaves of peony (*Paeonia officinalis* Retz) procured by Dr. H. A. ANDERSON near Crawfordsville, Indiana.

A good growth of mycelium and numerous sporing bodies were produced in plate cultures. The mycelial threads are slightly larger than those of other species of *Pestalozzia* studied. Pycnidial primordia arise usually by the compound meristogenous method (figs. 107, 108). As these bodies develop from the pycnidial stage to the open type, the formation of spores within (fig. 109), a

breaking open of the pycnidium, and its further development into what appears to be an acervulus may be seen.

Pestalozzia, species indet.; pure culture procured from Dr. G. P. CLINTON, New Haven, Connecticut, in November 1916. It had been isolated from dead maple (*Acer*) bark in October 1910.

This *Pestalozzia* was the most vigorous of all those cultured. Sporing bodies were produced in abundance. Two or 3 contiguous hyphae, or in some cases as many as 10 or 12, take part in the formation of the primordium. A few cells continue to swell and divide until a small mass is formed (fig. 110). They branch slightly (fig. 111) until a primordium of tissue-like type is formed. It is of compound meristogenous development, especially pronounced in cases where 10 or more hyphae take part. The young pycnidia-like bodies continue to develop, produce spores, then break open (fig. 112) and change into the acervulus form as in the previous species mentioned.

In *Pestalozzia* a condition is found that is quite distinct from any other acervulus-forming fungus studied, in that it first produces a sporing body which morphologically is a pycnidium. These pycnidia arise by any of the various modes previously described. The different species vary in the manner of development, but whatever the method there first appears a pycnidium which later breaks open and becomes acervulus-like.

PATELLINA Speg.

Patellina fragariae Stevens and Peterson; pure cultures procured of Dr. A. PETERSON, September 1916. It was also isolated from strawberries (*Fragaria chiloensis* Duschesne) from Centerville, Indiana, June 1916.

This fungus forms numerous sporodochia in concentric rings in plate cultures. These sporodochia arise in 2 rather typical ways. A few cells of a hypha divide into very short cells. These cells swell and bud, producing numerous branches in some cases (fig. 115), in other cases a tuft of 2 or 3 branches. These branches elongate slightly to form a pedicel (fig. 116), then branch at the tips and radiate to form a distinct urnlike body (fig. 117), within which a cushion or bed forms and gives rise to the conidiophores. The

sporodochia are usually produced singly by a simple meristogenous mode. In other instances sporogenous areas develop, and in these 1 or 2 cells, in each of the closely lying hyphae, branch profusely (fig. 113). Many of these branches unite into a mass and, without the formation of a definite pedicel and peridium (fig. 114), give rise to hundreds of conidiophores.

Patellina, species indet.; from a quince (*Cydonia vulgaris* L.) procured on the Champaign market, November 1917.

Strands of 6–20 hyphae are formed, and at some definite point within the strand a few adjacent cells branch; thus a tuft of a number of branches is formed (fig. 118). This tuft becomes slightly larger at the upper end by continued branching, while the lower portion constitutes a pedicel consisting of a few large branches, where a few hyphae enter into its formation. A more substantial closely formed pedicel or base is present if a larger number of hyphal branches are concerned in its origin (figs. 118, 119). The upper half or less becomes cuplike, and the outer hyphae curve inward as a superficial covering, while within conidiophores and conidia are formed. The type is compound meristogenous.

In *Patellina* the sporodochium develops in a very characteristic manner, arising by the meristogenous method. A single isolated mycelial thread gives rise to a very simple sporodochium. If a number of mycelial strands are crowded together, each branches characteristically, and the branches form a sporodochium of the compound type. The type of sporodochium varies with the type of base formed.

Volutella Tode

Volutella fructi S. and H.; procured from Mr. Wilmer G. Stover, Ohio State University. It was isolated by G. C. Meckstroth in the winter of 1916 from a fruit of apple (*Pyrus malus* L.) grown in western Ohio.

The sporodochia form in abundance in plate cultures, the primordium arising from a main hypha. A definite portion, 1, 2, or 3 cells in length, takes on a brownish color. These cells divide and some of them branch, usually from one side only (figs. 120,

124), forming a small tuft (figs. 120, 125), the branches of which elongate and then divide again, forming a larger tuft (figs. 121, 122). The first interwoven branches form a short pedicel. This cuplike structure may be regarded as the primordium of the sporodochium (fig. 123), from which a bed of short conidiophores is formed. The development is clearly simple meristogenous. Other larger bases are quite often formed symphogenously. Then hyphal branches from all adjacent mycelial threads interweave and anastomose, forming a black mass of sclerotial character from which hyphae arise and interweave into a cuplike bed from which conidiophores develop.

Volutella circinans (Berk.) Stevens and True; from a white globe onion (*Allium cepa* L.) at Urbana, Illinois, September 1917.

Primordia arise by either of 2 methods, simple meristogenous (fig. 126) or symphogenous (figs. 128–130). In most instances a black stroma-like mass of interwoven hyphae is formed symphogenously (figs. 130, 131), from which the sporodochium later develops. Setae may be found arising as hyphal branches (fig. 127).

Volutella is very similar to *Colletotrichum* and *Gloeosporium* in the origin of the sporing bodies; the meristogenous and symphogenous methods are both found. The principal distinction is in the formation of a more compact and usually larger base or subicle upon which the sporodochium is produced. The origin of this subicle if it is simple is usually meristogenous, but if a complex subicle is formed it arises symphogenously by the interweaving of numerous hyphal branches.

Epicoccum Link

Epicoccum, species indet.; isolated from a plate culture in which it appeared as a contamination in October 1917.

The sporing body of this fungus arises from 2 or more closely lying hyphae which produce erect branches from a rather localized area. Two or more such hyphae arise which branch in turn near their tips. These form a spreading tuft of conidiophores each bearing a conidium at its end. This is a simple form of sporodochium (fig. 132). The development is compound meristogenous.

PYCNIDIAL STAGE OF MELIOLA(?) CAMELLIAE (Catt.) Sacc.

This pycnidium was studied from herbarium material collected by Dr. F. L. STEVENS in Porto Rico. The fungus, which usually is reported as *M. camelliae* and is perhaps better known as "sooty mold" (48), is plainly not a true *Meliola*, as the genus is limited by recent writers (16, 40), and the position of its ascigerous stage has not been definitely determined.

A small pycnidium arises from a single cell of the main hypha or a single cell of a hyphal branch. The initial cell is usually an intermediate cell, but it may be a terminal one. In either case it divides by transverse and diagonal walls into 2, then 4 cells (fig. 133), and by swelling and dividing becomes an elliptical, finely reticulated, dark brown body (figs. 134–137). The mode is simple meristogenous.

These observations do not essentially disagree with the descriptions as given by ZOPF (53) and TULASNE (43), who studied early stages of the development of the pycnidium of the sooty molds.

Discussion

Two main methods of origin and early development are found in pycnidial formation, namely meristogenous and symphogenous. The meristogenous method resolves itself into 2 modes, simple (figs. 1, 2, 4, 5) and compound (figs. 18, 22, 37). In the simple mode the pycnidium develops from a single cell or a few adjacent cells of a single hypha. In the compound mode adjacent cells of 2 or more contiguous hyphae divide, swell, and sometimes branch, all of these then anastomosing freely to form a pseudoparenchymatous mass. Variations of these 2 modes are found in *Macrophoma citrulli* (figs. 39, 43, 47), *Coniothyrium pyriana* (figs. 69–71), *Septoria polygonorum* (figs. 76, 77), *Sphaeronaema fimbriatum* (figs. 50–52), and *Sphaeronaemella fragariae* (figs. 82–85).

The symphogenous method (figs. 48, 49, 54, 55) is less often found in the species studied. In this method of development, branching hyphae from main mycelial threads are directed toward a common point, loop back, and interweave to form a loose network which later becomes more close. The hyphae of this ball anastomose into a pseudoparenchymatous mass from which the pycnidium develops.

Table I gives the species and the methods of development found in each of the pycnidium-forming species. *Phoma herbarum*, *Phoma pirina*, *Phoma* from clover, *Phoma* from grape, *Sphaeronaema fimbriatum*, *Coniothyrium* species indet., *Septoria helianthi*, *Sphaeronaemella fragariae*, and a pycnidium of *Meliola*(?) *camelliae* show a simple meristogenous origin and development, and other methods of development seldom or never appear.

TABLE I

Species	Simple meristogenous	Compound meristogenous	Symphogenous
Phoma herbarum West	+	−	−
Phoma destructiva Plowr	+	+	−
Phoma pirina (Fries) Cooke	+	−	−
Phoma from clover	+	−	−
Phoma from grape	+	−	−
Phoma cichorii Passr	+	+	−
Macrophoma citrulli (B. and C.) Berl. and Vogl	+	+	+
Sphaeronaema fimbriatum (E. and H.) Sacc	+	−	−
Sphaeropsis malorum Pk	−	+	+
Sphaeropsis citricola McAlpine	+	+	−
Coniothyrium pyriana (Sacc.) Shel	+	+	−
Coniothyrium, species indet	+	−	−
Septoria polygonorum Desm	+	+	+
Septoria scrophulariae Pk	+	+	−
Septoria helianthi E. and K	+	−	−
Sphaeronaemella fragariae S. and P	+	−	−
Pycnidium of Meliola (?) camelliae (Catt.) Sacc	+	−	−

In *Phoma destructiva*, *Phoma cichorii*, *Sphaeropsis citricola*, *Coniothyrium pyriana*, and *Septoria scrophulariae* the pycnidial primordia arise by either the simple or compound meristogenous modes, the simple mode being the more common.

Macrophoma citrulli, *Sphaeropsis malorum*, and *Septoria polygonorum* give rise to their pycnidial primordia by either the symphogenous or meristogenous methods. In *Sphaeropsis malorum* the symphogenous method is the main one. The compound meristogenous method appears occasionally. In *Macrophoma citrulli* the simple meristogenous mode prevails, but the others are often found. In *Septoria polygonorum* the compound meristogenous mode is more often found, although a few primordia arise by the symphogenous method, and occasionally the simple meristogenous mode appears.

Variations of these 3 modes of development are found. No 2 species give exactly the same development. In symphogenous development a few branches from nearly hyphal strands may interweave near their ends, or intermingling hyphae may loop back, branch, and interweave or snarl into a knotlike mass. In the simple meristogenous mode the origin may be a single cell which swells and divides, or a number of cells that swell and divide simultaneously. In some cases branches arising from the dividing cells anastomose with the enlarging mass. In other cases branches arise from more distant cells of the same strand and take part in the development. In a few instances short budlike branches arise from a few cells of a hyphal strand, enlarge, divide into short cells, intertwine, and anastomose to form a pycnidium.

Neither have the acervuli-forming fungi in the Melanconiales been studied as to the early development of their sporing bodies, nor has the origin of the sporodochium been given special attention. SHERBAKOFF (37), in studies of *Fusarium*, describes a simple type of sporodochium found in cultures. TULASNE (43), STONEMAN (42), SOUTHWORTH (38, 39), WOLF (50, 51), and others figure and describe later stages of the development of acervuli, and the later development of sporodochia are referred to in the literature, but the subject is considered merely incidentally.

The fungi that form acervuli and sporodochia may be classed according to the manner of origin and development of the primordia on the same basis as the pycnidia-forming species.

In table II fungi of the Melanconiales and Tuberculariaceae that were studied are listed, indicating the method or methods of development of the primordia. In *Gloeosporium rufomaculans*, *Gloeosporium musarum*, and *Pestalozzia guepini* the primordia originate by the symphogenous method. In *Colletotrichum lagenarium* and *Volutella circinans* the symphogenous method prevails, although the simple meristogenous mode occurs occasionally. The compound meristogenous mode is well exemplified by *Pestalozzia palmarum*, *Pestalozzia* from peony and from maple. *Epicoccum* and *Pestalozzia* from maple have only the compound meristogenous mode. The simple meristogenous method of development appears in *Colletotrichum lagenarium*, *Pestalozzia palmarum*, *Pestalozzia*

from peony, *Volutella fructi*, and *Volutella circinans;* it also is at times observed in the development of isolated sporodochia of *Patellina fragariae*. This mode is more seldom found than either the compound meristogenous or symphogenous method in the development of acervuli and sporodochia.

TABLE II

Species	Simple meristogenous	Compound meristogenous	Symphogenous
Gloeosporium rufomaculans (Berk.) Thüm.	−	−	+
Gloeosporium musarum C. and M.	−	−	+
Colletotrichum lagenarium (Pers.) E. and H.	+	+	+
Pestalozzia palmarum Cke.	+	+	−
Pestalozzia guepini Desm.	−	−	+
Pestalozzia from peony	+	+	−
Pestalozzia from maple	−	+	−
Patellina fragariae S. and P.	+	+	−
Patellina from quince	−	+	−
Volutella fructi S. and H.	+	−	+
Volutella circinans (Berk.) S. and T.	+	−	+
Epicoccum, species indet.	−	+	−

The species of *Pestalozzia* studied present a type of sporing structure which is in need of further investigation in other genera and species. From the mature structure it has been classed as an acervulus, but from its origin and development it is not a true acervulus, for it arises as a pycnidium and opens to form an acervulus when mature. This may be called a pseudo-acervulus.

According to Potebnia (30) and Diedicke (10), some species of *Septoria* and *Ascochyta* have open-topped sporing bodies arising by the interweaving of hyphae to form a bed from which arises a peridium partially surrounding the inner sporing surface. Such a structure is designated by Potebnia as a pseudo-pycnidium.

Study of the origin and development of the sporing bodies of the many genera and species of the Sphaeropsidales and Melanconiales which have not yet been investigated will no doubt add to these 2 types.

In the pycnidia-bearing fungi studied the meristogenous method of development is the more prevalent, the symphogenous type seldom appearing. In species forming acervuli and sporodochia,

the tendency is toward the more complex methods, especially if *Pestalozzia* be regarded as belonging to the Sphaeropsidales. As was to be expected, in the pycnidial development no sexual organs, ascogenous hyphae, or nuclear fusions were observed.

Summary

1. Pycnidia originate and develop by 2 main methods, namely, meristogenous and symphogenous.

2. The meristogenous method resolves itself into 2 modes, simple and compound.

3. Variations of the meristogenous method are found, for example, in *Coniothyrium pyriana* and *Sphaeronaemella fragariae*.

4. The symphogenous method is less often found and is variable.

5. Acervuli arise in the same manner as do pycnidia, simple acervuli by the simple meristogenous mode, and complex ones usually by the compound meristogenous or symphogenous method.

6. Complex subicles usually arise symphogenously, although they may arise by the compound meristogenous mode.

7. Simple sporodochia, especially those appearing on single isolated strands, originate by the simple meristogenous method.

8. Complex sporodochia, with a large base or subicle, usually arise either by the compound meristogenous mode or symphogenously.

9. The pseudo-acervulus of the species of *Pestalozzia* studied arises and develops as a pycnidium which breaks open and appears like an acervulus.

10. The simple meristogenous development is the more often found in the Sphaeropsidales, while the compound meristogenous and symphogenous modes are the more usual in the Melanconiales and Tuberculariaceae.

I gratefully acknowledge the very helpful guidance of Dr. F. L. STEVENS throughout the preparation of this thesis, and I also wish to express my appreciation of suggestions and encouragement by Professor WILLIAM TRELEASE. Thanks are also due others who have kindly furnished material or suggestions.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

LITERATURE CITED

1. ANDERSON, P. J., The morphology and life history of the chestnut blight fungus. Commission for investigation and control of chestnut tree blight in Penn. Bull. 7. 1913.
2. ANDERSON, P. J., and RANKIN, W. H., *Endothia* canker of chestnut. N.Y. (Cornell) Agric. Exp. Sta. Bull. 347. 1914.
3. ARNAUD, GABRIEL, Contribution a l'étude des Fumagines. Ann. l'Ecole Nat. d'Agric. Montpellier. II. 9:239. 1910.
4. BAINIER, G., and SARTORY, A., Étude d'une espèce nouvelle de *Pestalozzia*. Annales Mycol. 10:433. 1912.
5. BAUKE, HERMAN, Beiträge zur Kenntnis der Pycniden. I. Nova Acta Leop. Carol. Deutsch. Akad. 38:443. 1876.
6. BREFELD, O., Bot. Unters. über Schimmelpilze. 4:122. 1881.
7. ———, Bot. Unters. a.d. Gesammtgebeit d. Mykologie 9: and 10:1891.
8. DEBARY, A., and WORONIN, W. W., Beiträge zur Morphologie und Physiologie der Pilze. 3:1870.
9. DEBARY, A., Comparative morphology and biology of the Fungi, Mycetozoa, and Bacteria. 1884. Engl. transl. 1887.
10. DIEDICKE, H., Die Abteilung Hyalodidymae der Sphaerioideen. Annales Mycol. 10:135. 1912.
11. EDGERTON, C. W., The physiology and development of some anthracnoses. BOT. GAZ. 45:367–408. 1908.
12. ———, The new fig diseases. Phytopath. 1:13. 1911.
13. EIDAM, Dr., Über Pycniden. Beibl. Tagebl. 49 Versamml. Deutsch. Naturforsch. 1876; rev. in Just's Bot. Jahresber. 4:176. 1876.
14. ———, Über Pycniden. Bot. Zeit. 35:60. 1877.
15. FISCHER, ED., Beitrag zur Kenntnis der Gattung *Graphiola*. Bot. Zeit. 41:745. 1883.
16. GAILLARD, A., Le genre *Meliola*. Paris. 1892.
17. GIBELLI, G., and GRIFFINI, L., Sul polimorfismo della *Pleospora herbarum* Tul. Ricerche Lab. Bot. Crittogamica Pavia; rev. in Just's Bot. Jahresber. 1:125. 1873.
18. HALSTED, B. D., and FAIRCHILD, D. G., Sweet potato black rot. Jour. Mycol. 7:1–11. 1891.
19. HESLER, L. R., Black rot, leaf spot, and canker of pomaceous fruits. N.Y. (Cornell) Agric. Exp. Sta. Bull. 379. 1916.
20. HIGGINS, B. B., Contribution to the life history and physiology of *Cylindrosporium* on stone fruits. Amer. Jour. Bot. 1:145. 1914.
21. ———, Life history of a new species of *Sphaerella*. Mycol. Centralbl. 4:187. 1914.
22. JAMIESON, CLARA O., *Phoma destructiva*, the cause of a fruit rot of the tomato. Jour. Agric. Research 4:1. 1915.
23. KLEBAHN, VON H., Beiträge zur Kenntnis der Fungi Imperfecti. I–II. Mycol. Centralbl. 3:49, 97. 1913.

24. LEININGER, H., Zur Morphologie und Physiologie der Fortpflanzung von *Pestalozzia palmarum* Cooke. Centralbl. Bakt. II. 29:3. 1911.
25. LEVIN, EZRA, The leaf spot disease of tomato. Mich. Agric. Col. Exp. Sta. Tech. Bull. 25. 1916.
26. MERCER, W. B., On the morphology and development of *Phoma Richardiae* Mycol. Centralbl. 2:244. 1913.
27. NICHOLS, MARY A., The morphology and development of certain Pyrenomycetous Fungi. BOT. GAZ. 22:301. 1896.
28. PIERCE, NEWTON B., A disease of almond trees. Jour. Mycol. 7:66. 1892.
29. PLANCHON, LOUIS, Influence des milieux sur les Dématiées. Ann. Sci. Nat. Bot. VIII. 11:1. 1900.
30. POTEBNIA, A., Beiträge zur Micromycetonflora Mittel-Russlands. Annales Mycol. 8:42–93. 1910.
31. REDDICK, DONALD, The black rot disease of grapes. N.Y. (Cornell) Agric. Exp. Sta. Bull. 293. 1911.
32. SACCARDO, P. A., Sylloge Fungorum.
33. SCHNEGG, H., Zur Entwicklungsgeschichte und Biologie der Pykniden, sowie der Schlingenmycelien und Hyphenknäuel. Centralbl. Bakt. II. 43:326. 1915.
34. SCHOSTAKOWITSCH, W., Über die Bedingungen der Conidienbildung bei Russtbaupilzen. Flora 81:362. 1895.
35. SHEAR, C. L., Cultural characters of the chestnut blight fungus and its near relatives. U.S. Dept. Agric. Bur. Pl. Ind. Circ. 131. 1913.
36. SHEAR, C. L., and WOOD, ANNA K., Studies of fungous parasites belonging to the genus *Glomerella*. U.S. Dept. Agric. Bur. Pl. Ind. Bull. no. 252. 1913.
37. SHERBAKOFF, C. D., Fusaria of potatoes. N.Y. (Cornell) Agric. Exper. Sta. Mem. no. 6. 1915.
38. SOUTHWORTH, E. A., Anthracnose of cotton. Jour. Mycol. 6:100–105. 1891.
39. ———, Ripe rot of grapes and apples. Jour. Mycol. 6:164. 1891.
40. STEVENS, F. L., The genus *Meliola* in Porto Rico. III. Biol. Monographs. 2:7. 1916.
41. STEWART, V. B., The leaf blotch disease of horse chestnut. Phytopath. 6:5–19. 1916.
42. STONEMAN, BERTHA, A comparative study of the development of some anthracnoses. BOT. GAZ. 26:69–120. 1898.
43. TULASNE, L. R. and C., Selecta Fungorum Carpologia. 2:1863.
44. VAN DER BIJL, PAUL A., A study of the "dry-rot" disease of maize caused by *Diplodia zeae*. Dept. Agric. Div. Bot. and Pl. Path. Sci. Bull. no. 7. 1916. Pretoria.
45. VOGES, E., Über die Pilzgattung *Hendersonia* Berk. Bot. Zeit. 68:87. 1910.

46. VON TAVEL, FRANZ, Beiträge zur Entwickelungsgeschichte der Pyrenomyceten. Bot. Zeit. 44:825. 1886; transl. in Jour. Mycol. 5:53. 1889.
47. ———, Vergleichende Morphologie der Pilze. Jena. 1892.
48. WEBBER, HERBERT J., Sooty mold of the orange and its treatment. U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. 13. 1897.
49. WENNER, JOHN T., A contribution to the morphology and life history of *Pestalozzia funerea* Desm. Phytopath. 4:375. 1914.
50. WOLF, FREDRICK A., The perfect stage of *Actinonema rosae*. BOT. GAZ. 54:218. 1912.
51. ———, Some fungous diseases of the prickly pear, *Opuntia Lindheimeri* Engelm. Ann. Mycologici. 10:113. 1912.
52. ZOPF, W., Die Pilze, in SCHENK'S Handbuch der Botanik. 4:1890.
53. ———, Die Conidien Früchte von *Fumago*. Nova Acta Leop. Carol. Deutsch. Akad. 40:1878.

EXPLANATION OF PLATES XVII–XXII

The drawings were made with a Leitz camera lucida and a Leitz one-twelfth oil immersion lens or a no. 6 objective. The scale appears on the plates.

PLATE XVII

Phoma

FIG. 1.—*P. herbarum* West: 4-celled stage of pycnidial primordium of simple meristogenous origin.

FIG. 2.—Slightly later stage.

FIG. 3.—Stage similar to fig. 2.

FIG. 4.—Young pycnidium with light colored rostrum where ostiole will form.

FIG. 5.—Mature pycnidium and spores formed by simple meristogenous development.

FIG. 6.—*P. pirina* (Fries) Cooke: few-celled stage; simple meristogenous origin.

FIG. 7.—Slightly later stage.

FIG. 8.—Many-celled pseudoparenchymatous stage with branches protruding from mass.

FIG. 9.—Irregular mass from which pycnidium develops.

FIGS. 10, 11.—Few-celled stages in origin of which 2 or more hyphae are involved; compound meristogenous origin.

FIG. 12.—Similar to fig. 11, but slightly older.

FIG. 13.—Primordium in which a 3-parted hyphal strand is involved.

FIGS. 14–16.—*P. destructiva* Plowr.: beginning stages in pycnidial development; simple meristogenous origin.

FIG. 17.—Slightly later stage.

FIGS. 18, 19.—Compound meristogenous development in which 2 or more hyphae are involved.

FIG. 20.—Development near end of hypha with branches budding from mass.

FIG. 21.—Irregular meristogenous development.

FIG. 22.—Typical compound meristogenous development with 3 parallel hyphae involved.

PLATE XVIII

FIGS. 23, 24.—*Phoma* from clover: beginnings of pycnidia.

FIG. 25.—Slightly older stage with a few short budding branches.

FIGS. 26, 27.—Later stages with one end enlarging slightly.

FIG. 28.—*Phoma* from grape: mycelial threads with different stages of pycnidial development.

FIG. 29.—Beginning stage: simple meristogenous.

FIGS. 30–32.—Later stages.

FIG. 33.—Mature pycnidium.

FIG. 34.—*P. cichorii* Passr.: early stage in development; short cells formed which divide and branch.

FIG. 35.—Early stage in which is much branching.

FIG. 36.—Medium stage in simple meristogenous development.

FIG. 37.—Typical compound meristogenous development.

Macrophoma

FIG. 38.—*M. citrulli* (B. and C.) Berl. and Vogl.: 2-celled stage in origin of pycnidium, from drop culture; simple meristogenous development.

FIG. 39.—Same 12 hours later.

FIGS. 40, 41.—Early stages of other pycnidia, from drop culture; slight variation from simple meristogenous type in that many branches are involved.

FIGS. 42, 43.—Early stages in which much branching takes place; drop culture.

FIGS. 44, 45.—Early stages from Petri dish culture.

FIGS. 46, 47.—Later stages.

PLATE XIX

FIG. 48.—*M. citrulli* (B. and C.) Berl. and Vogl.: symphogenous development in which branches from a number of main strands interweave.

FIG. 49.—Later stage in which a winding of the hyphae and cell division have taken place forming a pseudoparenchymatous mass.

Sphaeronaema

FIGS. 50–52.—*S. fimbriatum* (E. and H.) Sacc.: early stages of pycnidium in which a hypha coils, branches, and divides to form knotlike mass.

Sphaeropsis

FIG. 53.—*S. malorum* Pk.: Early stage in symphogenous development in which branches *a*, *b*, *c* interweave near their ends to form a ball.

FIG. 54.—Interwoven hyphae in early stage of symphogenous development.

FIG. 55.—Slightly later stage.

FIGS. 56, 57.—*S. citricola* McAlp.: very early stages in origin of simple meristogenous development of pycnidia.

FIGS. 58, 59.—Slightly later stages.

FIGS. 60–63.—Later stages with short hyphae branching from masses.

FIGS. 64–66.—Unusual examples of developments in which more than one hypha is involved; compound meristogenous.

Coniothyrium

FIGS. 67, 68.—*C. pyriana* (Sacc.) Shel.: early stages in development.

FIGS. 69, 70.—Later stages in which numerous branches from dividing mass are involved.

FIG. 71.—Pseudoparenchymatous mass from which a pycnidium arises.

PLATE XX

FIG. 72.—*Coniothyrium* species from laboratory air: early stage showing short cells and short branches as origin.

FIG. 73.—Slightly later stage in which cells and branches from main hypha divide into short cells and anastomose.

FIG. 74.—Stage slightly more developed than fig. 73; slight variation from simple meristogenous type.

Septoria

FIG. 75.—*S. polygonorum* Desm.: beginning stage of simple meristogenous development.

FIG. 76.—Development in which original hypha and numerous branches are involved.

FIG. 77.—Pseudoparenchymatous primordial mass formed by symphogenous method.

FIGS. 78, 79.—*S. scrophulariae* Pk.: early stages in compound meristogenous development.

FIG. 80.—*S. helianthi* E. and K.: early stage in beginning of simple meristogenous development.

FIG. 81. Later stage.

Sphaeronaemella

FIG. 82.—*S. fragariae* S. and P.: early stage of simple meristogenous development.

FIG. 83.—Later stage in which branches and original hypha have anastomosed to form a mass on one side of main strand.

FIG. 84.—Later stage in which mass surrounds main strand.

FIG. 85.—Late stage which has developed by a winding and dividing of hyphal branches from a few cells upon one side of main strand.

Gloeosporium

FIG. 86.—*G. rufomaculans* (Berk.) Thüm.: early stage of meristogenous development; this method rarely occurs.

FIG. 87.—Side view of an acervulus formed by symphogenous method: compressed so that cushion-like bed of conidiophores is pulled away from basal hypha.

FIG. 88.—*G. musarum* C. and M.: top view of partially developed acervulus; development symphogenous.

Colletotrichum

FIG. 89.—*C. lagenarium* (Pers.) E. and H.: very early stage in which acervulus originates in a few short branches from a single hypha.

FIG. 90.—Numerous budding branches from a few cells of one hypha forming beginning of an acervulus; simple meristogenous.

FIG. 91.—Early stage in development of acervulus.

FIG. 92.—Primordium in which a few branches are involved.

FIG. 93.—Early stage in symphogenous development: hyphal branches loop and interweave in this mode.

FIG. 94.—Same as fig. 93, 24 hours later.

FIG. 95.—Beginning stage of symphogenous development.

FIG. 96.—Later stage.

PLATE XXI

Pestalozzia

FIG. 97.—*P. palmarum* Cke.: *a*, very early stage in meristogenous development, a few cells divided and swelling; *b*, later stage.

FIGS. 98, 99.—Pycnidia with young spores developed within.

FIG. 100.—Primordium of compound meristogenous origin.

FIG. 101.—Young pycnidium at stage just before opening.

FIG. 102.—Pycnidium opening and extruding spores: following this stage cuplike interior formed, longer conidiophores develop, and body becomes a pseudo-acervulus.

FIGS. 103, 104.—*P. guepini* Desm.: early stages in symphogenous development.

FIG. 105.—Later stage; pycnidia of this species form spores within and later break open as pseudo-acervuli.

FIG. 106.—*Pestalozzia* from peony: meristogenously developed pycnidial mass.

FIG. 107.—Early stage in symphogenous development.

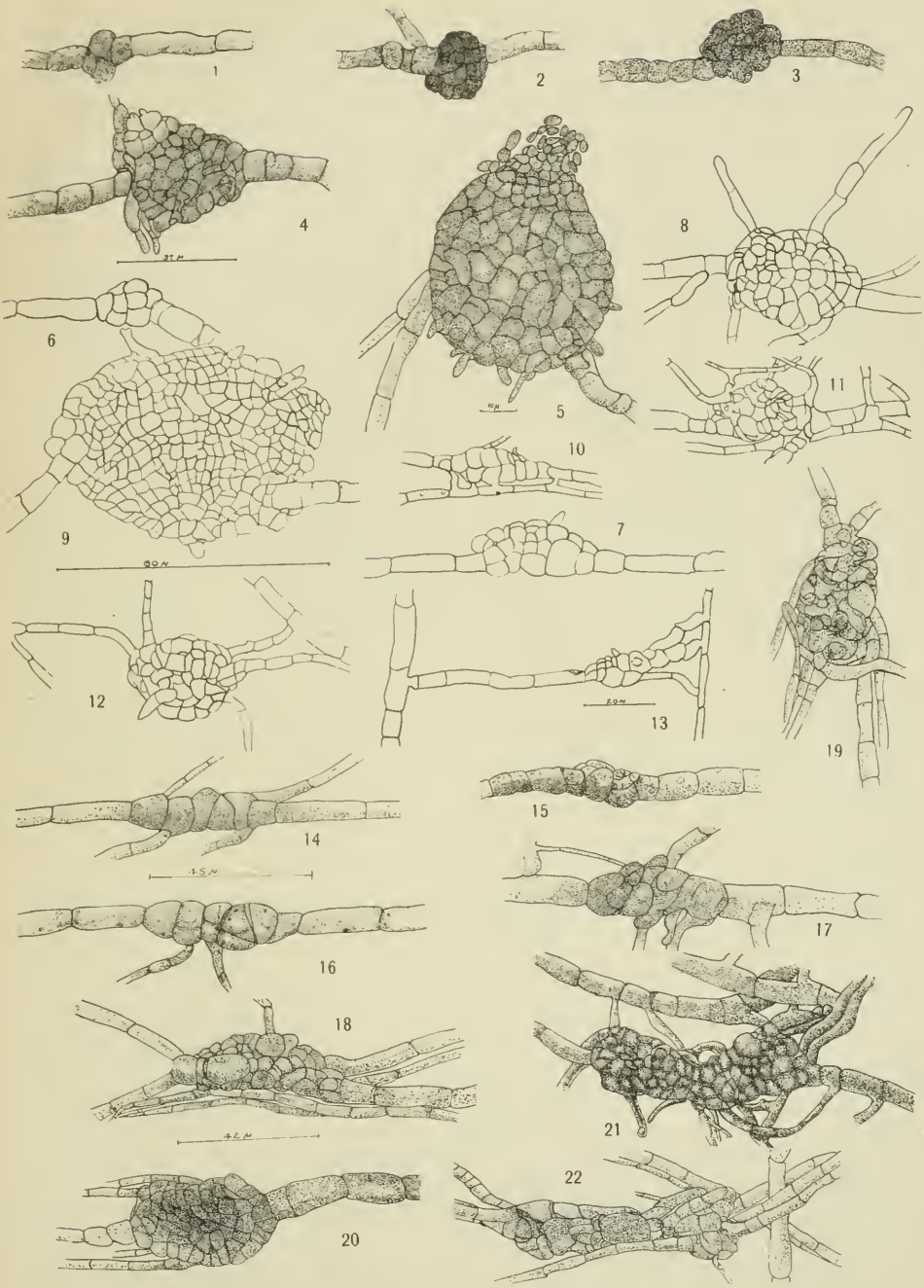
FIG. 108.—Later stage.

FIG. 109.—Pycnidium which has opened, surface view.

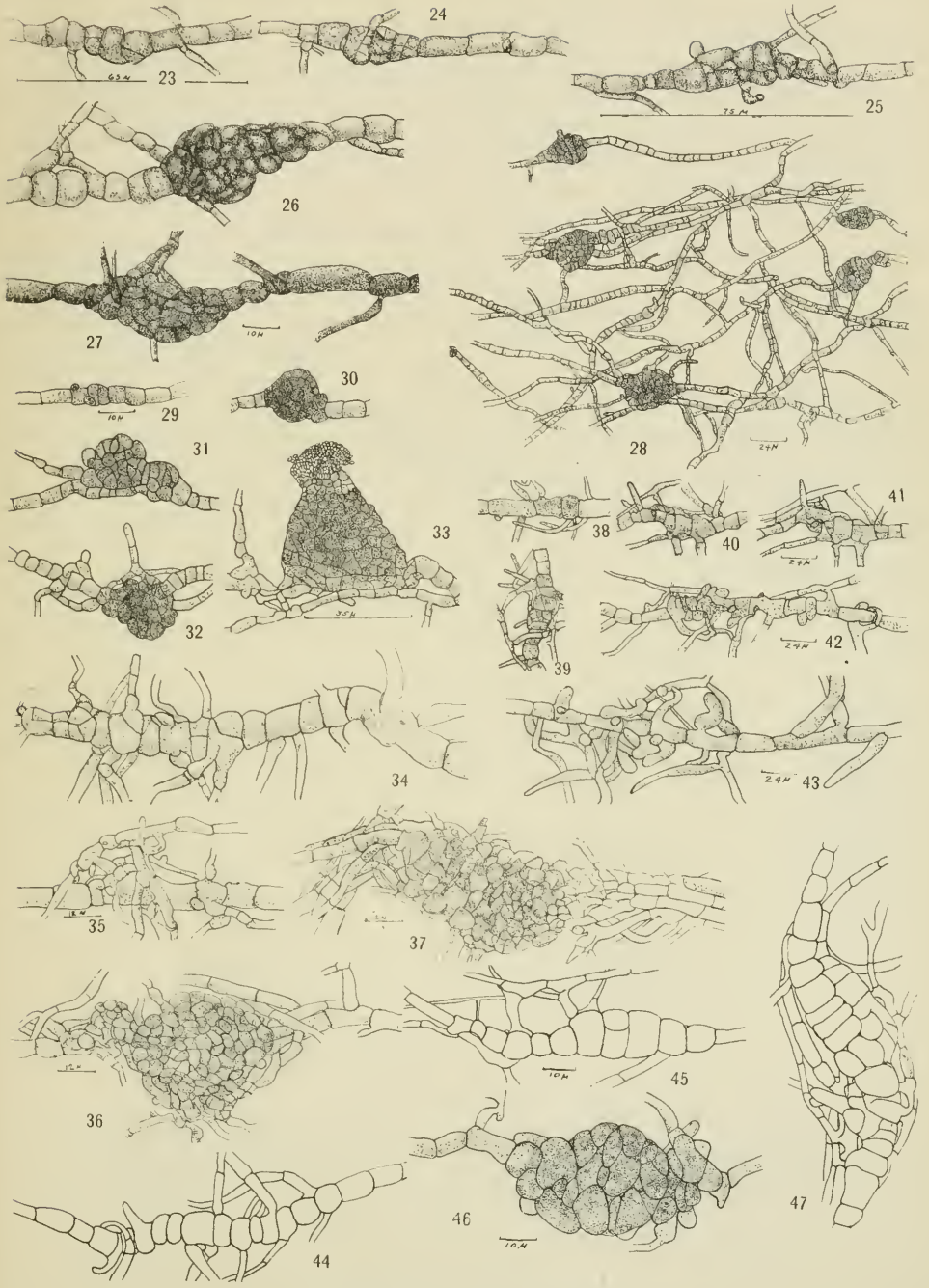
FIG. 110.—*Pestalozzia* from maple: compound meristogenous development from a number of parallel hyphae.

FIG. 111.—Later stage.

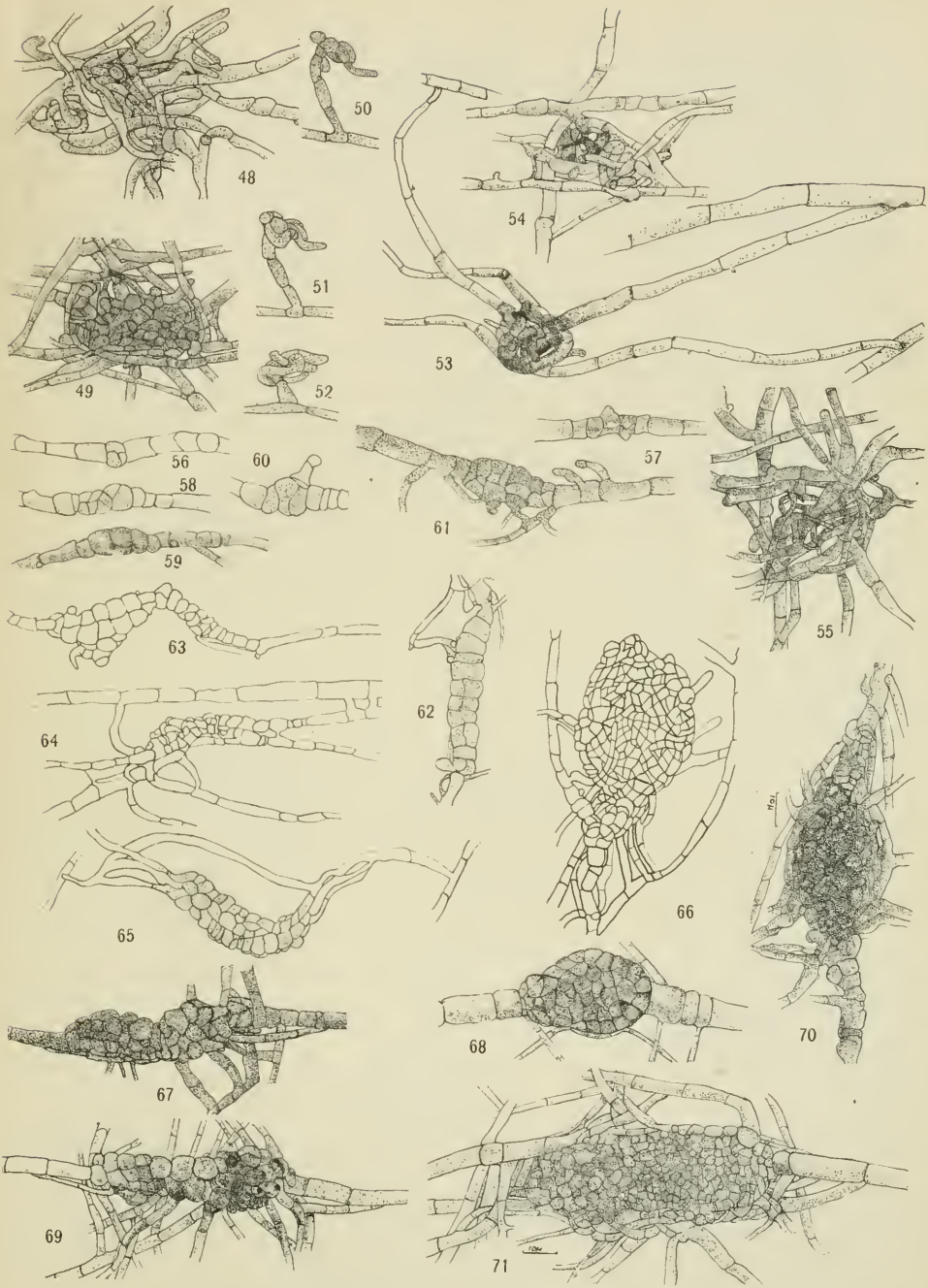
FIG. 112.—Young pycnidium breaking open on one side: spores show within.



KEMPTON on PYCNIDIUM

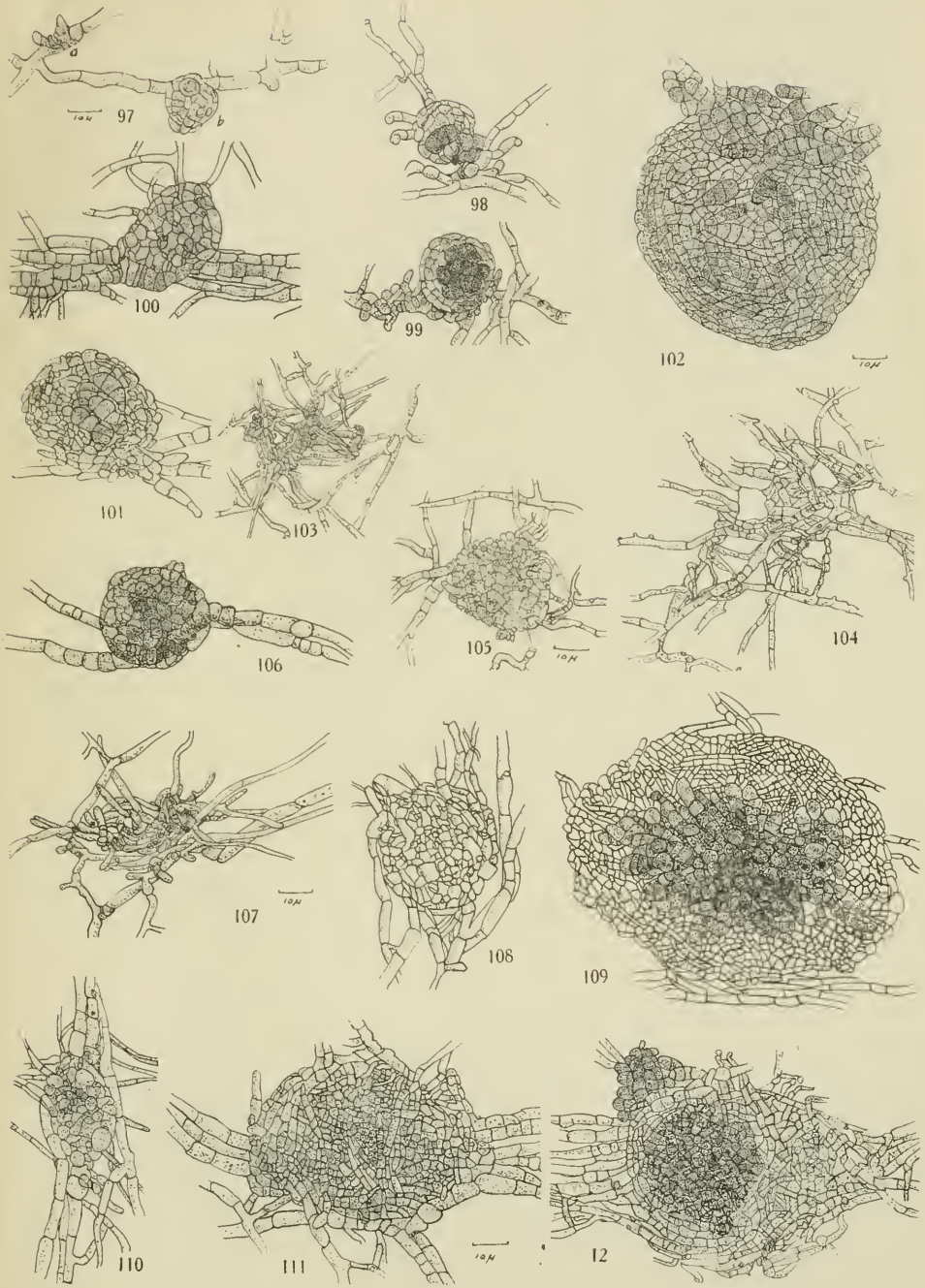


KEMPTON on PYCNIDIUM

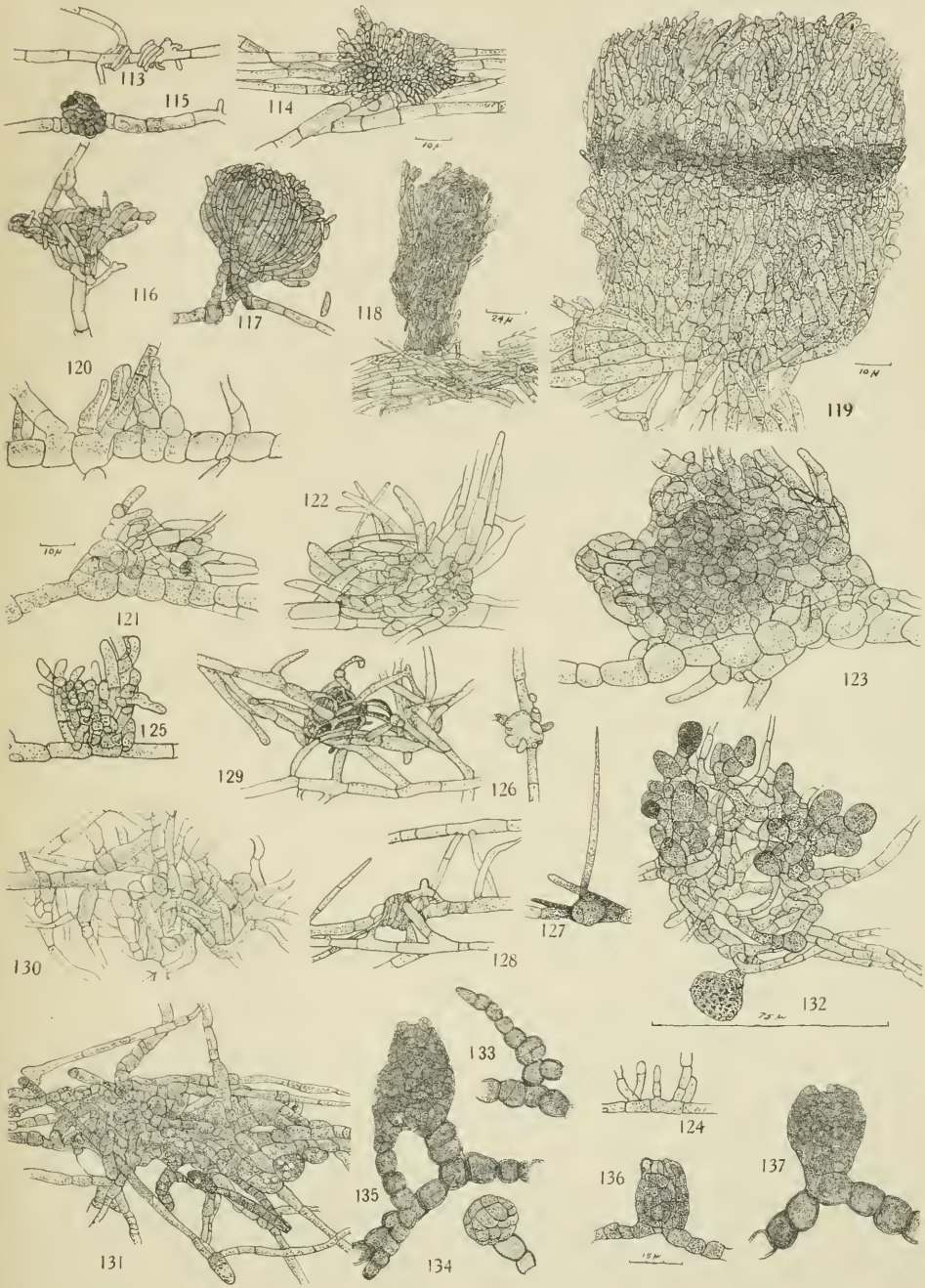


KEMPTON on PYCNIDIUM

KEMPTON on PYCNIDIUM



KEMPTON on PYCNIDIUM



KEMPTON on PYCNIDIUM

PLATE XXII

Patellinia

FIG. 113.—*P. fragariae* S. and P.: single strand, with characteristic branching, from sporogenous area.

FIG. 114.—Compound meristogenously developed sporodochium produced from a number of hyphae, as fig. 113.

FIG. 115.—Simple meristogenous development with branches arising from a few cells.

FIG. 116.—Later stage in simple meristogenous development.

FIG. 117.—Simple, meristogenously developed, mature sporodochium.

FIG. 118.—*Pestalozzia* from quince: sporodochium of compound meristogenous development; spore bearing area develops from cuplike top.

FIG. 119.—Another view of sporodochium similar to fig. 118.

Volutella

FIG. 120.—*V. fructi* S. and H.: very early stage in simple meristogenous origin of a sporodochium; few cells branch to form base.

FIG. 121.—Slightly later stage.

FIG. 122.—Medium stage.

FIG. 123.—Fully developed base or subicle.

FIG. 124.—Same as fig. 120: smaller hypha.

FIG. 125.—Later stage than fig. 124.

FIG. 126.—*V. circinans* Stevens and True: simple meristogenous origin of subicle in which a cell swells and branches by budding.

FIG. 127.—Hypha and branches: one branch has developed into a seta.

FIG. 128.—Beginning of symphogenous development of a subicle.

FIG. 129.—Later stage.

FIG. 130.—Many branches interweaving to form a subicle.

FIG. 131.—Complex symphogenously developed subicle.

Epicoccum

FIG. 132.—*Epicoccum*, species indet.: compound meristogenous development of simple sporodochium with young spores.

Meliola(?)

FIG. 133.—Pycnidium with *M.*(?) *camelliae:* 4-cell stage developed from single cell within a hyphal branch.

FIG. 134.—Many-celled stage apparently developed from end cell of hypha.

FIG. 135.—Mature pycnidium developed from single cell within hypha.

FIG. 136.—Same as fig. 135.

FIG. 137.—Slightly different pycnidium from figs. 133, 135.

ECOLOGY OF TILIA AMERICANA

I. COMPARATIVE STUDIES OF THE FOLIAR TRANSPIRING POWER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 253

JAMES E. CRIBBS

(WITH THIRTEEN FIGURES)

Introduction

Up to the present time transpiration studies have been conducted almost exclusively with potted plants, or upon plants which were growing under controlled conditions. This is due to the fact that most of the investigation has been purely physiological in its aims; and since the factors influencing this process are numerous, they can evidently be more definitely calculated in controlled habitats than in natural ones.

It has been the investigator's aim to carry this line of experimentation into the field, in an endeavor to determine what differences occur in the relative foliar transpiring power of a certain species when growing in different environments. It was at once recognized that the complication of factors which influence transpiration is considerably increased, and the precision with which they may be measured and the relative values to be attributed to each are perhaps less exact than when such investigation is made under greenhouse or laboratory conditions. Nevertheless, there are certain problems which of necessity must be worked out under field conditions, especially when we wish to determine the ecological value or relationship of the environment.

For this investigation *Tilia americana* was chosen because it, perhaps more than any other of our tree species, grows under a wide range of environmental conditions. This is especially true when we consider its unusual ability (as a member of a mesophytic forest) to surmount moving dunes which chance to advance upon it. This ability of *Tilia* to persist on the moving sands brings it

into a set of varying conditions which are widely different from those of its normal mesophytic environment, and affords an excellent opportunity to investigate certain ecological and physiological features which are brought under particular stress because of the abrupt change in growth conditions.

Five stations for investigation were selected on the dunes near Miller, Indiana, and will be designated here as *A*, *B*, *C*, *D*, and *E*.

FIG. 1.—Station *A*, showing *Tilia* on forested complex, associated with *Psedera*, *Smilacina*, *Acer*, and *Prunus*.

Studies were conducted also at station *F* in a mesophytic forest on morainic clays in western Pennsylvania, that a comparison might be had for different soil conditions.

Station *A* (fig. 1) is located on an established dune complex which has advanced to a state of mesophytism in regard to both the tree and herbaceous vegetation. It is well sheltered from the strong lake winds, and is not exposed to the intense light and the accompanying high temperatures of the open sand areas.

Station *A* lies deeper in the complex than *B* (fig. 2), which is situated at the base of a forested dune and is not more than 15 m.

from the edge of a blowout. The conditions are very similar at *A* and *B* so far as the humus is concerned. This has attained a thickness of approximately 5 cm., which is indicative of a relatively long period of stability. The presence of a well defined humus is correlated with a very rich development of herbaceous undergrowth and tree seedlings.

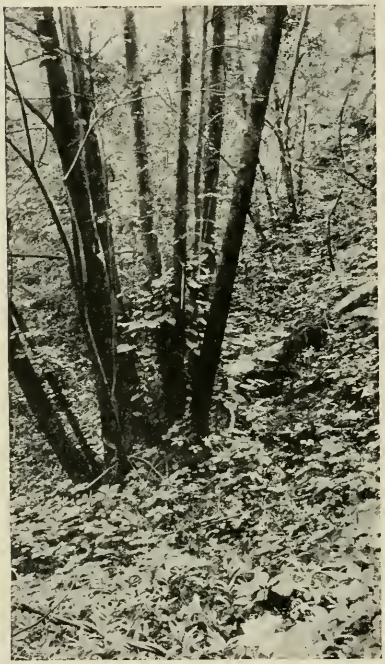


FIG. 2

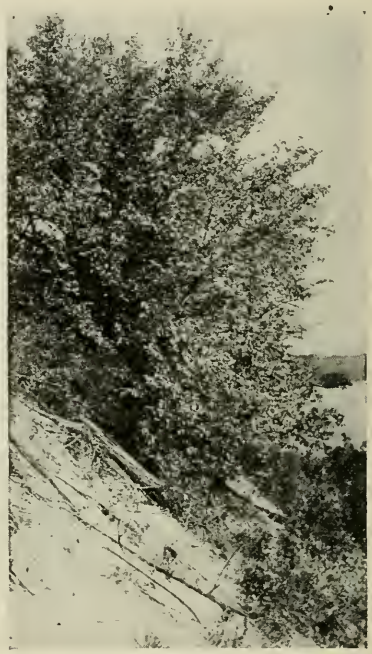


FIG. 3

FIGS. 2, 3.—Fig. 2, station *B*, showing position of *Tilia* at base of well established dune and with same plant associates; fig. 3, station *C*, where *Tilia* is located on edge of blowout; humus being broken up by sand movement, and access of wind and light much greater than at *A* or *B*.

Station *C* (fig. 3) is located on a west-facing slope on the edge of a blowout where there is a ready access to strong winds. The exposure to light is much greater here than at either *A* or *B*, especially in the afternoon when the sun's rays fall vertically upon the slope, while the stations in the forested complex are deeply shaded. The free sand movement has destroyed the humus and

with it a large part of the accompanying herbaceous vegetation, thus developing a situation of greater openness and more intense exposure.

Station *D* (fig. 4) is located on the lee slope of an advancing dune. In most respects this position is more xerophytic than that at *C*, for there is a complete absence of humus and herbaceous

FIG. 4

FIG. 5

FIGS. 4, 5.—Fig. 4, station *D*, with *Tilia* on lee slope of advancing dune; sand advance rapid at this point, and absence of humus further inhibits herbaceous development; plant associates: *Cornus stolonifera*, *Ammophila arenaria*, and *Prunus virginiana;* fig. 5, station *E*, showing *Tilia* located on crest of high dune and exposed to most desiccating conditions found in dune environments.

undergrowth. The incipient rays are strongly reflected from the open sand, giving a very high light intensity and considerable increase in temperature. Being on a south-facing slope, the greatest exposure occurs in the late forenoon, followed early in the afternoon by shade which is continuous until evening.

Station *E* (fig. 5) represents the most exposed habitat to be found on the open sands, as it is situated about 25 m. above the

lake on the crest of an eroding dune which is exposed to wind from all points of the compass. The water content is less at this point than at lower levels and the light intensity becomes the greatest, the sun striking the station about 8:00 A.M., from which time it remains directly exposed until evening. The absence of humus and the exposure to wind combine to give a high sand mobility, which means a most unstable kind of habitat.

FIG. 6.—Station *F*, showing *Tilia* in mesophytic forest on clay soil; chief plant associates: *Osmorhiza*, *Adiantum*, *Caulophyllum*, *Aralia*, and *Actaea*.

Station *F* (fig. 6) is located in a forest, the chief tree members of which are *Acer*, *Liriodendron*, *Castanea*, and *Prunus serotina*. The herbaceous undergrowth includes such mesophytic species as *Aralia racemosa*, *Adiantum pedatum*, *Osmorhiza longistylis*, *Viola pubescens*, etc. The humus at this station has a depth of about 5 cm. and the underlying soil is a mixed morainic drift.

Methods

The cobalt chloride paper method was employed to determine the relative transpiring power. This method, first used by STAHL (15), has been improved and employed by subsequent investigators and is undoubtedly the one in present use which is best adapted for work in the field. Whatman's filter paper no. 30 was used throughout this work and was treated with a 3 per cent solution of cobalt chloride and prepared in accordance with the method described by LIVINGSTON and SHREVE (10). Preliminary tests were made with the plain paper and the tricolor slips of LIVINGSTON and SHREVE, and as essentially the same coefficients

were obtained the tricolor paper was discarded as being more difficult to prepare and less easily handled in the field. The plain paper has the additional advantage of being somewhat slower, and is thus more satisfactory when used at stations on the open sands, for the change in color was found to take place so quickly that even the paper with longer time values was frequently difficult to read. The probability of error arising from short time periods was largely eliminated by increasing the number of readings from the usual 5 to about 8 or 10, and the time recorded in each instance was the average of all readings made.

The paper was applied to the leaf surface by means of the clip devised and described by LIVINGSTON (8). At each of the stations leaves were chosen which were about 1 m. above the ground; readings were taken from the same relative position on the different leaves; and so far as possible the same set of leaves was employed in each subsequent day's work. Records were taken from 2 leaves at each station, usually at hourly intervals, beginning as soon as there was sufficient light to observe the color change and continuing until darkness prevented further reading. Records given here were taken from the abaxial (stomatal) side only.

The indirect method of determining the color change over the standard evaporating surface, as suggested by BAKKE (1), was used during the second summer's work, thus making it unnecessary to take the standard cup of LIVINGSTON and SHREVE into the field.

Various devices were tried for heating the hygrometric paper to force off the water of crystallization, for considerable difficulty was experienced because of the prevalence of stong lake winds, so a special lamp was devised and used for this purpose. The chief difficulty with the acetylene lamp was to get a steady flame for a long period of time. That which was finally employed consisted of a railroad lamp, with a round wick, which had an oil capacity of about 0.5 liter. A tubular piece of tin was fashioned so that it had a transverse dimension of about 8 cm. and a length of 23 cm. This was fitted to the base by means of 3 guides so as to leave a space of about 2 mm. below for ventilation. Into the

upper end was set a small tin cup such as may be obtained at any hardware store, and over this a screen lid was placed for use when working in a high wind. For ventilation above the flame, a ring of small holes was cut about 2.5 cm. below the level of the inserted cup. This lamp was found to be readily controlled, burns alcohol or kerosene equally well, can be used successfully in a strong wind, and burns continuously for 48 hours or more without refilling or adjustment.

Relative humidity was calculated hourly by means of the sling psychrometer, such as is in use by the United States Weather Bureau, and the wet bulb depression was referred to a standard table as given by MARVIN (12) to obtain the relative humidity values.

Soil temperatures were recorded for depths of 2 dm. and 4 dm. by means of a centigrade thermometer mounted on a cylindrical piece of hickory which was well adapted for inserting into the sand. Atmospheric temperature records were taken and recorded hourly during each day of experimentation.

Soil samples were collected at the different stations on the days when these were worked and the moisture computed on the basis of dry weight. From these results growth water was calculated by the equation GW = SW − WC, in which SW = total soil water and WC = the wilting coefficient. Samples were taken from depths of 2 and 3 dm. and dried for 6 days at a temperature of 100° C.

The wilting coefficient of the soil was computed by the centrifuge method of BRIGGS and MCLANE (4). The moisture equivalent was obtained directly and the wilting coefficient derived from this by the equation of BRIGGS and SHANTZ (3), $\frac{ME}{1.78} = WC$.

The evaporating power of the air was recorded by means of the porous clay cup of LIVINGSTON (7). Hourly readings were readily secured by mounting the atmometer on a graduated burette tube which was refilled when the water column fell to a point about 25 cm. below the level of the cup.

Field work was conducted under diverse weather conditions to discover to what extent the varying of such factors as relative

humidity, wind, light, temperature, etc., might influence the rate of transpiration in the field, and to compare its relative influence in the different environments. In each instance readings were taken at stations *A*, *B*, and *C* on the same day. The same may be said for stations *D* and *E*. Fig. 7 represents the records of relative transpiring power, temperature, relative humidity, and evaporation for *A*, *B*, and *C* on July 21, 1918. As in the following graphs, the scale to the left is for the index of foliar transpiring

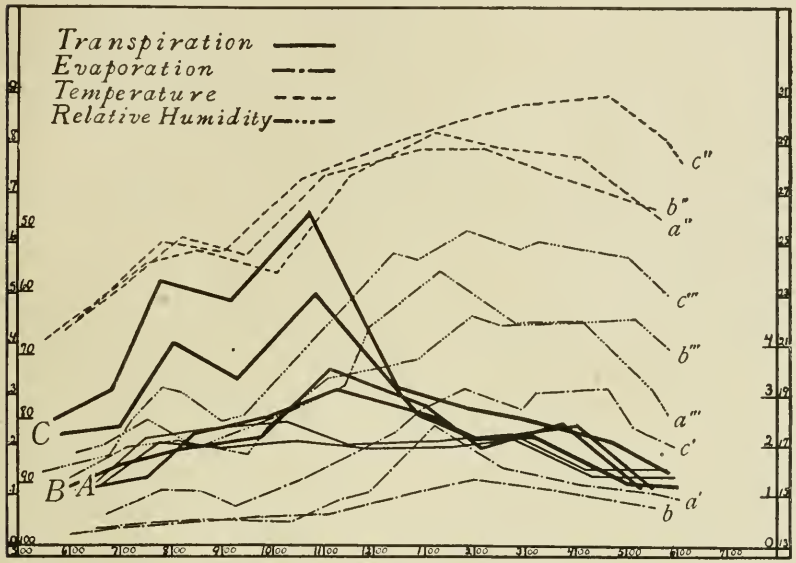


FIG. 7.—Graph giving transpiration, temperature, evaporation, and relative humidity curves of stations *A*, *B*, and *C* for July 21, 1918; scale similar in all following graphs (see text).

power; the inner one to the left is for relative humidity; the inner one to the right is for evaporating power of the air and is expressed in cubic centimeters per hour; and the scale to the right records the atmospheric temperature in degrees centigrade. Time is indicated at the bottom of each graph.

It will be noted that there is a close parallelism between the curves representing temperature, evaporation, and relative humidity. This is in fact what would be expected, as these factors are

closely interrelated. Accompanying the opening of the stomata in the morning, there is a rise in the transpiration indices, and their curves follow closely those of the factors just mentioned until about 11:00 A.M., when a marked divergence occurs. The transpiration curve declines rapidly from a maximum at this time, while the curves representing relative humidity, temperature, and evaporation continue to rise until about 2:00 P.M., following which there is a gradual decline. This feature is little in evidence in the graph of station *A*, but becomes more so in those of *B* and *C;* that is, the divergence becomes more and more conspicuous with an increase in exposure of habitat. This same feature will be found to recur with greater prominence when we consider the data of stations *D* and *E* on the open sand.

There has been considerable discussion concerning the interpretation of this sudden decline: LLOYD (11), following his investigation of stomatal action in *Fouquieria* and *Verbena*, concluded that the earlier view that stomatal movement controlled the transpiration rate was not entirely true, for there may be an increase in the transpirational loss for some time after the maximum opening; and there was found to be frequent decrease and subsequent increase in the afternoon, with little or no accompanying stomatal movement. RENNER (13) advanced the concept of "saturation deficit" to explain this behavior in transpiration, and LIVINGSTON and BROWN (9) in the following year discussed this behavior under the "incipient drying" theory. These are essentially the same concept, namely, that following the rise in the transpiration rate which accompanies the stomatal opening in the morning a point may be reached when the turgidity of the mesophyll cells of the leaf becomes sufficiently reduced to increase the surface tension of the water films in the walls. A check in the molecular diffusion of water from the cells follows. The increased concentration of the cell contents would then exert additional pull on the water in the translocating system, and a restoration of turgor may then follow without visible wilting occurring in the leaf. The length of time elapsing before the restoration of turgor depends largely upon the evaporating power and temperature of the atmosphere, also upon the relative humid-

ity of the air, and in some cases upon growth water. In recording the daily transpiration march in *Tilia* this saturation deficit was found to be very characteristic, especially at times when the relative humidity is low. It is also induced more readily when the growth water is near the zero point.

There is another condition, however, under which a very similar behavior in the graph is noted and may very easily be confused with the preceding. This would be especially liable to happen when one is employing the porometer and cobalt chloride paper only, as was done in the work of TRELEASE and LIVINGSTON (16). This cause lies in a sudden change in relative humidity. Thus if there is a sudden increase in relative humidity the effect on the transpiration index is to depress it. Such depressions may be detected from saturation deficits by an accompanying lowering in the curve of evaporation, for when it is due to a deficit the evaporation curve will be high. Inasmuch as a depression in transpiration caused by an increase in relative humidity is not accompanied by a closure of stomata, according to LLOYD (11), the porometer of course would continue to record the relative stomatal opening, and a divergence in the graphs would result. Instances of this appeared a number of times during the experiments, and were very marked on the occurrence of transient showers.

The sudden depression at 8:00 A.M. in graphs of station *C* (fig. 7) is due to this influence. It will be noticed that there is a corresponding increase in relative humidity, with a lowering of temperature and evaporation power. The behavior at this time is definitely attributed to the passing of a thundershower some distance to the south. It is interesting to note that the influence is more pronounced at station *C* than at *A* or *B*, especially as regards transpiration and evaporation. The drop in evaporation at *C* is attributed mostly to a sudden period of calm which had less effect at *A* and *B* because of their more sheltered position. The greater effect in the relative transpiration curve at *C* is probably to be interpreted as the result of a greater susceptibility of the leaf to a change in relative humidity when transpiring at a high rate.

The curves in fig. 8 were plotted from readings taken at station *D* on September 2. One of the outstanding features of this graph is the parallelism in all the curves. It will be observed that the maxima of transpiration coincide with those of temperature, evaporation, and relative humidity; and that there is an absence, or at least almost a complete absence, of an afternoon saturation deficit. In both of these respects this graph differs from the preceding one. The difference was found to be related to the atmospheric conditions. The evaporating power of the air

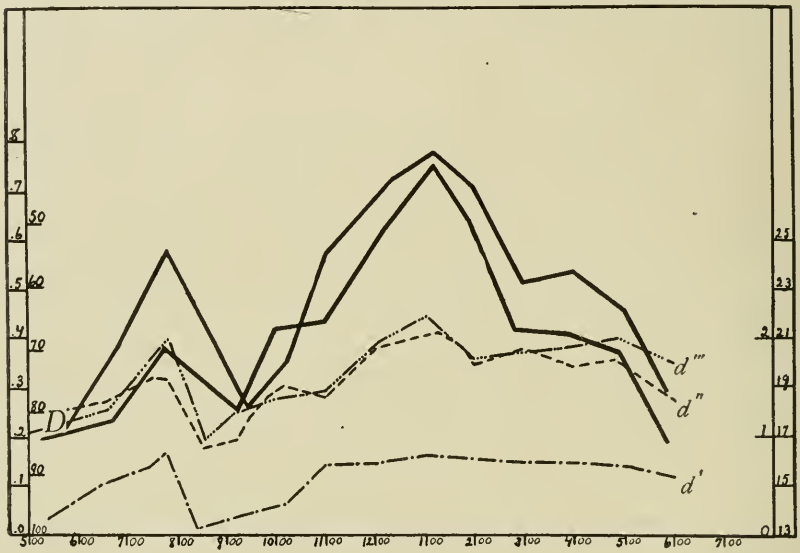


FIG. 8.—Graph plotted from data taken at station *D* on September 2, 1918; parallelism of curves and absence of saturation deficit due to high relative humidity.

was low throughout the day, never reaching 1 cc. per hour, while ordinarily at station *B* it became 2 cc. or more per hour by 2:00 P.M.; the temperature was low and the humidity high. This combination of factors in the field was always found to favor parallelism of curves and an absence of a saturation deficit. Under such conditions the maxima usually occur later in the day than when the deficit is developed.

At 7:30 A.M. there occurs a sudden fall in the transpiration indices which resembles the deficit drop. This was due to a sud-

den shower which began at 7:40 and lasted until 8:20. If the stomatal behavior is similar in *Tilia* to that found by LLOYD (11) to exist in *Fouquieria*, *Verbena*, and *Ampelopsis*, a porometer curve, if such had been made, would probably have continued to rise from 8:00 to 10:00 A.M. and would have given the type of curve that is obtained when a true deficit occurs. The evaporation curve alone shows that this was not a deficit depression, and one could safely infer the same from the much higher maximum that immediately follows; but if such a drop had occurred near midday, the second higher mode may not have occurred and a fall identical with that of the saturation deficit may have resulted. It will be noted that, notwithstanding the low evaporation rate, the transpiration index is quite high. This was found to be true for positions on the open sand, although under the same atmospheric conditions on humus the index was always much lower. This is probably largely due to the greater light intensity in the former position, and to a certain degree to higher temperatures.

It may be interesting to note the following atmospheric conditions in their general relation to the transpiration indices for this particular day: 5:00–7:20 A.M., partly cloudy; 7:20–7:40, cloudy; 7:40–8:20, rain; 8:20–9:30, cloudy; 9:30–10:00, clearing; 10:00–12:30 P.M., sun; 12:30–3:00 P.M., cloudy; 3:00–6:00 P.M., clear, but station shaded. From these data it will be seen that the drop from 1:00 to 3:00 P.M. was probably due to the sudden cloudiness of that period, which may have caused sufficient closing of the stomata to effect a depression. There is also a small drop in temperature and relative humidity at this time which would also have their influence, although not recorded by the atmometer. It should be said, however, that the white cup is less influenced by light changes than is the leaf (LIVINGSTON 6), and this was found to be most noticeable on the open sand when the index of transpiration was high.

One of the most striking relations that appeared in these comparative studies was found in the readings taken at the stations located on humus and those on the open sand during the latter part of the summer. As previously stated, stations *A* and *B* are in a forested complex which has a well developed humus

and an abundant herbaceous undergrowth, and *C* is on the edge of the same complex, where it is being destroyed by a blowout. These stations, when compared with *D* and *E*, stand out in contrast by their greater mesophytism. This is true in regard to the texture of their foliage and its richness; but during the months of August and September the *Tilia* complex rapidly undergoes a change which is very noticeable in the vegetation and is conspicuous in the relative transpiration indices. This change is initiated by an early reduction of the soil moisture to the wilting coefficient, evidently because of the heavy vegetation the sands are supporting and the excessive transpiration rates caused during this period by the highly desiccating atmospheric conditions. Stations *D* and *E* show, during this same period, a higher growth water content on the exposed dunes; and the abscission which is carried on rapidly in the *Tilia* complex is entirely unnoticed here until much later. The greater soil moisture on the open sand as compared with the pine and oak dune stages has been pointed out in the work of FULLER (5), and is seen to be similar for the *Tilia* complex. Fig. 9 emphasizes this relationship. The data for stations *A*, *B*, and *C* were taken on August 26, and that for station *E* on August 11. Although the 2 sets of readings were not taken at the same time, the atmospheric conditions on the 2 days were practically identical. Both days were sunny throughout, and the general parallelism of temperature, evaporation, and relative humidity was rather unusual. Curves for these factors were plotted for stations *A* and *E* only.

The transpiration indices of *A*, *B*, and *C* were all very low, that of *C* being slightly higher than *A* or *B*. They rose slowly in the morning from a low point and reached a low maximum at 8:00–8:30 A.M. A deficit occurred then because the soil moisture had reached the wilting coefficient, and the indices remained low throughout the day, rising slowly in the evening as the leaves regained their turgor. Visible wilting occurred about 10:30 A.M. at these stations, and was sufficient to cause stomatal closure. The temperature, evaporation, and relative humidity are seen to have remained high, reaching a maximum about 3:00 P.M., after which they declined rapidly.

A very different behavior occurred at station *E*, where the exposure is more intense. The growth water here was found to be 0.679 per cent at 2 dm. and 1.054 per cent at 3 dm., but the leaves remained turgid throughout the day, and there was no visible wilting. The transpiration index at 6:00 A.M. was quite high, and rose rapidly during the morning to a maximum at 12:00 noon. Here a saturation deficit was developed and a sudden

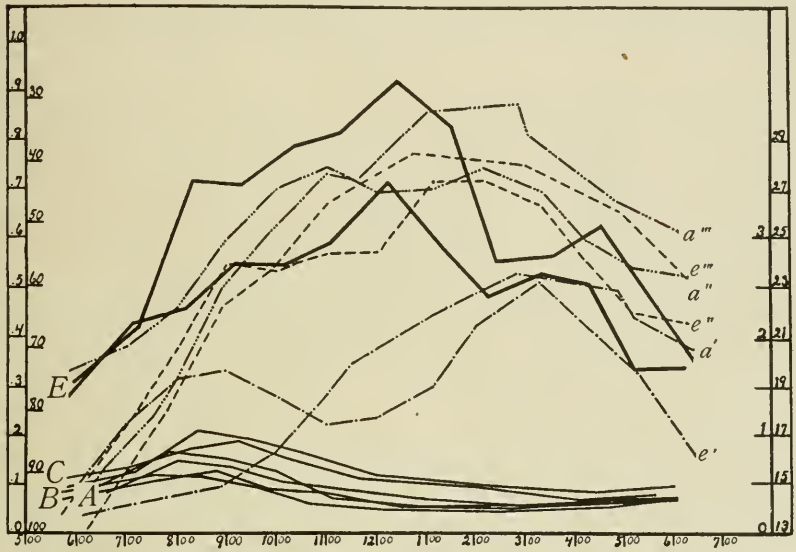


FIG. 9.—Graphs for stations *A*, *B*, and *C* on August 26 and station *E* on August 11, 1918; high transpiration index occurs on open sand with afternoon saturation deficit, while indices for humus stations remain low, due to visible wilting.

depression occurred until about 3:00 P.M., which was followed by a second low mode at 4:30.

This curve is a very typical one for a clear day at this position, in that there is a rapid rise in the morning to a high maximum, and a following clearly marked depression due to a saturation deficit, but no visible wilting occurs; then a second low mode in the afternoon about 4:00 P.M., followed by a rapid decline with the closing of the stomata in the evening. The morning maximum occurs from 9:00 to 12:00, unless disturbed in some way by

such influences as thundershowers and sudden shifting of wind to or from the lake with accompanying atmospheric changes in temperature, relative humidity, etc.

The usual pronounced saturation deficit that occurs on the exposed sands is shown in fig. 10, which is a graph of readings taken at station *E* on July 27. There was a very rapid rise to a maximum at 9:30 A.M., at which time the water loss presumably

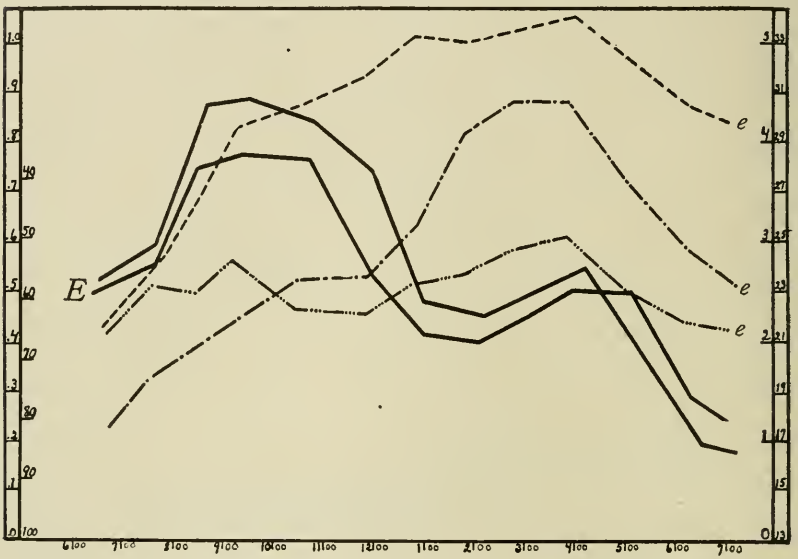


FIG. 10.—Graph plotted for station *E* on July 27, showing typical saturation deficit developed in open dune environments on clear days with temperature high or relative humidity low.

equaled the maximum translocating ability of the plant under the conditions. Then as a deficiency was created in the cells by an excessive loss, a drop in the index occurred until the turgor was regained. A second low mode occurred at 4:00 P.M., a feature recurring in all records taken at stations *D* and *E*, in which an appreciable deficit was developed. Although the decline in the relative transpiration index was considerable in the early afternoon, there was no visible wilting.

Fig. 11 is a composite graph in which the curve for each station is plotted from the average of all the readings taken. This figure shows the relative transpiring power of *Tilia* in the different dune environments, and it will be seen that there is a very pronounced increase in the index of transpiration when considering the stations in their order from the mesophytic to the more xerophytic habitats.

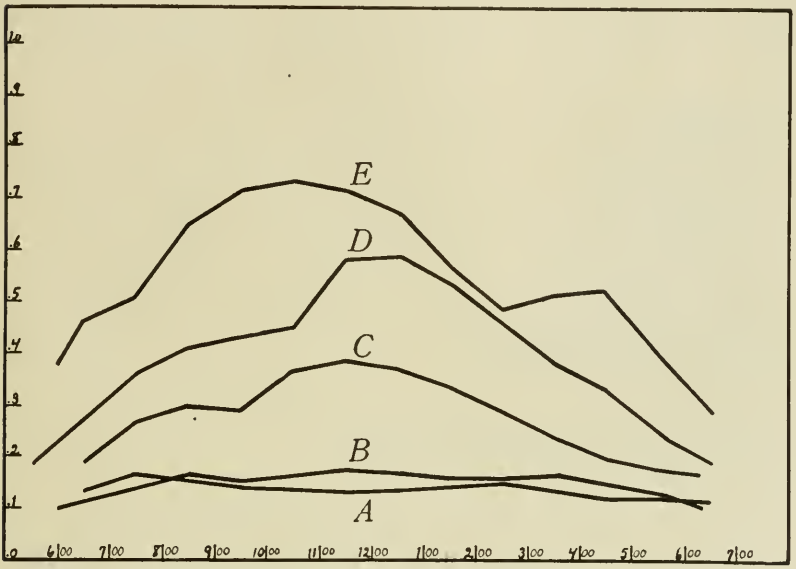


Fig. 11.—Composite graph of all transpiration readings taken at stations *A*, *B*, *C*, *D*, and *E*, showing increase in transpiring power accompanying increased exposure of habitat.

These curves being averages, and hence not recording daily variations, do not give to best advantage the typical daily curve. The occurrence of the maximum about 12:00 noon in the more mesophytic situations, however, and an earlier occurrence from 9:00 to 11:00 A.M. on the open sands, is noticeable even in the average graphs, as is also the earlier morning rise characteristic of the exposed stations.

Fig. 12 includes averages of all the different factors taken in connection with the dune transpiration studies. The unit spaces at the top of the graph have the following values: evaporation

5 cc. per unit; transpiration 0.1; relative humidity 15 per cent; soil temperature 7° C.; atmospheric temperature 10 per cent; and growth water 1 per cent per unit. From these it will be seen that the conditions existing at stations *A* and *B* are closely similar; but from *B* to *E* the stations represent a graded series of habitats as regards these factors, just as clearly as they do when we consider their comparative positions in the vegetative cycle. This graded variation found in the dune environments is much more pronounced than that for different situations on clays, which will

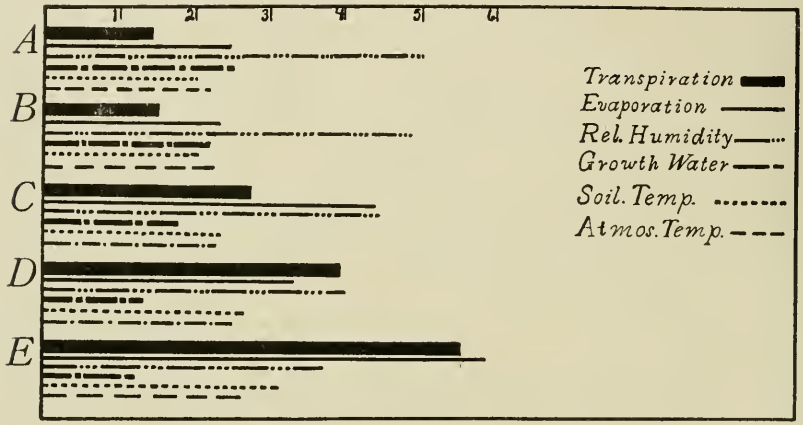


FIG. 12.—Comparative graph illustrating conditions of transpiration, etc., for the 5 dune environments; close gradation of factors evident here; increase in transpiration accompanying decrease in growth water is characteristic of dune habitats.

be mentioned only briefly in this paper. The increase in relative transpiring power that occurs with an increase in exposure of habitat is very evident.

Concerning evaporation, the only variation occurs at station *C*. Here the water loss is considerably more than at *D*. This is because of the greater access of wind at *C*, which is located on the edge of a blowout, while *D* is on a protected lee slope where only south and southeast winds have access.

The average relative humidity decreases rapidly from *A* to *E*, and is almost parallel with the increase in the transpiration index. Variation in humidity has been considered the most poten-

tial single factor influencing the transpiration index (BRIGGS and SHANTZ 2), and so far as considered in these studies it has been found to be a factor of the first order. It is not always the controlling one, however, for there are frequent variations in the daily march which owe their appearance to other causes. Temperature, stomatal movement, and growth water, for instance, at any given time may become the dominating factor, only to be succeeded as the conditions vary by some other which for the time being replaces it.

The proportional increase in the soil temperature over that of atmospheric temperature from *A* to *E* is of interest, and it may safely be inferred that high soil temperatures have a prominent part in the maintenance of the water supply, and hence indirectly concern the transpiration rate. Until more accurate quantitative measurements are made upon the effect of increase of temperature of the soil upon the water absorbing power of roots, however, the quantitative influence of this factor can only be speculated. Such work as has been done by RYSSELBERGHE (14) on the effect of temperature changes on the rate of osmotic transfusion through semipermeable membranes leads to the conclusion that the much higher soil temperature, as of *E* over station *A*, must be one of the factors which enable the exposed plants to take over water from the soil with sufficient rapidity to withstand the much higher transpiration loss.

One of the noteworthy features of the dune studies appears in the relation of the growth water to transpiring power. It may be seen from the figure that there is a low average of growth water at all stations, that for *A* being only about 2.46 per cent and that for *E* 1.25 per cent. While it is true that the highest average is at *A*, the greater percentage here is due to the higher content during the earlier summer months. During the latter part of July and August, however, the water available for plant absorption decreases more rapidly than on the open sand, and the frequent drop in soil moisture to the wilting coefficient produces a period of stress and leads to early abscission. Meanwhile the growth water on the moving complex remains practically constant, especially at a depth of 3 dm. or below, where it is about

1.25 per cent or more. This greater constancy is attributed to the moving mulch of dry sand which breaks the continuity of surface films in the soil particles, thus preventing rapid evaporation. This ability of a plant to maintain a higher transpiration index with a growth water content of 1.25 per cent than the same species does with 20 per cent or higher, such as is commonly true for clays, indicates that the amount of growth water has but little relation to the transpiration index so long as the soil moisture content remains above the wilting coefficient. This gives a somewhat unusual aspect to the question of mesophytism and the part played by soil water as a factor in plant growth. So far as *Tilia* is concerned, it produces more vigorous vegetative structures, which retain their activities later in the summer, when growing in open situations on sand than when in the forested dune complex; and the factor of greatest importance seems to be, not the average growth water of the soil, but whether the available moisture repeatedly falls below the wilting coefficient. So long as it is above that point, although it is by only a very small percentage, the normal activities, including high transpiration, are carried on.

There is one point concerning this relation which needs investigation, however, namely, the relative extensiveness of the root systems in the two situations. I am inclined to the idea that the ability of *Tilia* to develop adventitious roots when covered by an advancing dune may enable such individuals to draw their water supply through a root system the absorptive surface of which is greater in proportion to the amount of foliage than is true of this species when growing in the forest complex. Another point of probable difference is that the individuals on the open sand may obtain a considerable portion of their water from a greater depth than do those on the humus; but the extent to which the root systems persist when submerged by advancing dunes has never been worked out.

As indicated by the averages shown in fig. 11, the situation at *E* is distinctly more xerophytic than at *A*. Every fact recorded in the experimentation points to this conclusion. The size,

general shape, and texture of the leaves at station *E* are also distinctly xerophytic, but it has been noted that notwithstanding this the transpiration index is higher, a fact not at all in accordance with the behavior of desert plants so far reported, for they are characterized by a low transpiration index, as pointed out by several workers. With this fact in view BAKKE (1) has suggested the foliar index of relative transpiring power as a test for the mesophytism of a plant, as follows: "As a result of the preliminary study, it may be suggested that plants exhibiting a diurnal foliar transpiring power of less than 0.30 may be regarded as xerophytes, while those exhibiting indices above 0.70 may be considered mesophytes." It may be seen at once that the behavior in *Tilia* is the reverse of that common to desert plants, and hence an application of this test would lead to confusion. I believe with BAKKE, however, that with certain reserve this method may be used as a fair indicator of mesophytism, provided two precautionary measures are taken: first, that the species under consideration be chosen in its normal environment and not in an abnormal or forced one; and second, that hourly readings be taken for at least two full days and that the relative humidity and temperature conditions be carefully employed in calculating the results. This latter precaution is necessary because of the great variation in the index at different times of the day, in the first place, and because of the wide variation of the indices at any particular hour on two successive days when the relative humidity or temperature has undergone considerable change.

The development of a xerophytic leaf under unusual conditions of exposure has been found in *Tilia* to result in a leaf less effective in preventing water loss than are desert types. This may be attributed to a lagging of the effect behind the causal factors. On the other hand, such lagging may be considered as not occurring, for there is always a favorable balance in water relations which permits a greater vegetative activity than would be possible in desert plants, and becomes possible on the open sands only because of a sufficient and permanent growth water throughout the growing season.

At station *F* there is considerable difference in the environment, and the factors accompanying it, from that of the dune series. It has already been noted from the introductory description that the undergrowth is composed of a more shade-requiring assemblage than even the most mesophytic positions found on the established dune complex. The humus is slightly more developed, but the soil underlying it is very different, and unlike in the forested dune habitats the growth water was always adequate to support an abundant and diversified vegetation, and never reached the wilting coefficient. The average available water at 2 dm. was 19.40 per cent and the minimum 12.45 per cent. Thus if growth water is inducive of a high transpiration, one would expect to find it here, but the average transpiration at this station for 5 complete days' readings was only 0.16, an index practically identical with that of station *A* of the *Tilia* forested complex where the growth water averaged 2.5 per cent.

The same tendency displayed by the dune graphs to show curves with a single mode recurs here where the mesophytism is greater. The maximum power of transpiration comes later in the day, commonly from 12:00 to 2:00 P.M., and is more frequently coincident with the maxima of temperature, evaporation, and relative humidity. This relation is shown in fig. 13, which is a graph of station *F* on June 14. At this time there was a growth water content of 26.74 per cent and a relatively low humidity. The temperature was low, while the evaporation was high when compared with the transpiration, higher than commonly found in dune environments. The morning rise of the transpiration index was very slow and the maximum reached was not very high. On this particular day it was clear until about 2:00 P.M. with increasing cloudiness through the afternoon, followed by showers at 8:00 P.M. Although it was clear during the forenoon the station was shaded throughout the period.

The fact that direct sun upon the leaves in a habitat of this sort almost always leads to a rapid increase in the transpiration rate would suggest that the low light intensity of the densely shaded forest is one of the chief factors leading to the low average commonly found there.

Summary

1. Cobalt chloride standardized paper was found well suited for comparative studies in the relative transpiring power of leaves in the field.

2. The daily march of transpiration in *Tilia* was found to vary greatly for the same leaf on different days. This variation was found to be influenced by relative humidity, temperature, light intensity, soil moisture, and presumably by soil temperature.

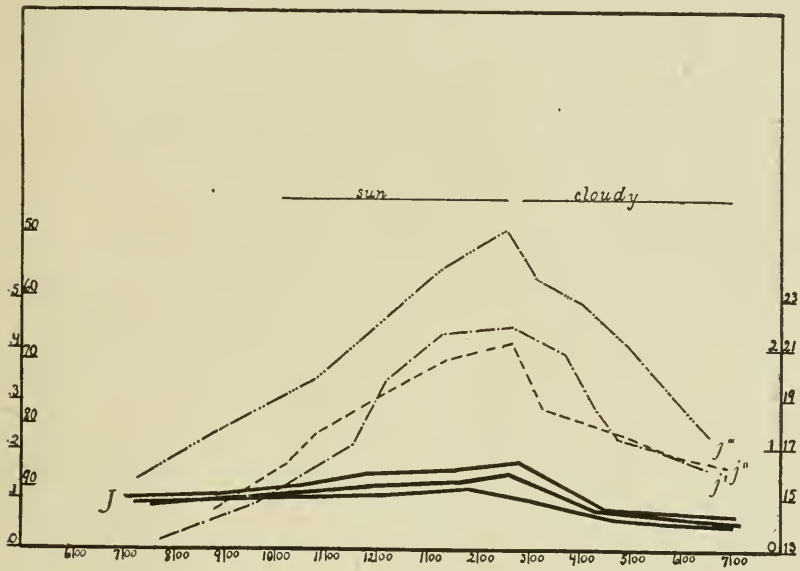


FIG. 13.—Typical graph illustrating low transpiration indices occurring in morainic mesophytic forest where growth water is always high; note low transpiration for a day with so low a relative humidity.

3. The foliar transpiring power of *Tilia* was found to increase in dune environments from an index of 0.15 on the forested *Tilia* complex to 0.55 in the most exposed situations on the open sand. In habitats between these extremes the transpiration power was found to be directly proportional to the relative exposure.

4. The morning rise in the daily march is more rapid on the open sand, where it reaches a maximum 1 to 2 hours earlier than in forested situations.

5. In the forested complex the curve representing relative transpiration tends to develop a single mode about midday, and this maximum tends to coincide with the maxima of temperature, relative humidity, and evaporating power of the air. The greater the mesophytism and relative humidity the more striking becomes this tendency.

6. In the most exposed situations on the open sand the relative transpiration maximum usually appears about 10:00 A.M., while the maximum temperature; relative humidity, and evaporating power occur from 2:00 to 4:00 P.M. This divergence from parallelism is due to the development of a saturation deficit, which appears successively earlier as the exposure of the habitat increases. The more mesophytic the habitat the less noticeable becomes this deficit, until it disappears entirely, especially on humid days.

7. The foliar transpiration index is influenced less by wind currents than is the porous cup atmometer.

8. Transpiration curves showing a saturation deficit depression usually develop a second mode about 4:00 P.M., which is, so far as noticed, always lower than the mode preceding the deficit depression.

9. Bimodal transpiration curves have been found to be due either to a saturation deficit or to a sudden increase in relative humidity, although lesser depressions may result from fluctuating temperature or intensity of light.

10. No evidence of visible wilting occurred in *Tilia* on the open sand at any time during the summer, although the so-called "incipient drying" was a common feature of the stations throughout this period. On the forested complex, however, visible wilting occurred during the first week in August because the vegetation was so dense that the water content of the soil was reduced to the wilting coefficient quite early.

11. The average mesophytism on the *Tilia* complex is considerably greater than on the open sand, the growth water averages being 2.5 and 1.25 per cent respectively; but the open positions are practically constant in their water relations, while the forested complex represents a decreasing mesophytism as the summer

advances, the growth water in the spring being higher than at any other time.

12. The amount of growth water in the soil apparently has very little influence on the transpiration index, unless it is reduced to the wilting coefficient. It might be argued that a low growth water is the cause of the saturation deficit depression, but there is evidence that it is due rather to the inability of the translocating system to conduct water to the leaves with sufficient rapidity to offset the transpiration loss, and not to a slowing up of the absorption rate. This is substantiated by the occurrence of the typical deficit in readings on *Tilia* when the growth water was greater than 20 per cent. The drop that occurs when the soil moisture falls to the wilting coefficient is more permanent and is due to stomatal movement which accompanies visible wilting.

I wish to express my grateful appreciation of the encouragement and suggestions given by Dr. GEO. D. FULLER, of the University of Chicago.

COLLEGE OF EMPORIA
EMPORIA, KAN.

LITERATURE CITED

1. BAKKE, A. L., Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. Jour. Ecol. 2:145–173. 1914.
2. BRIGGS, L. J., and SHANTZ, H. L., Daily transpiration during the normal growth period and its correlation with the weather. Jour. Agric. Res. 7:155–212. 1916.
3. ———, The wilting coefficient and its indirect determination. BOT. GAZ. 53:20–37. 1912.
4. BRIGGS, L. J., and MCLANE, L. W., The moisture equivalent of soils. U.S. Dept. Agric. Bur. Soil Bull. 45. 1907.
5. FULLER, G. D., Evaporation and soil moisture in relation to the succession of plant associations. BOT. GAZ. 58:193–234. 1914.
6. LIVINGSTON, B. E., Light intensity and transpiration. BOT. GAZ. 52: 417–438. 1911.
7. ———, Atmometry and the porous cup atmometer. Plant World 18: 21–30, 51–74, 95–111, 143–149. 1915.
8. ———, The resistance offered by leaves to transpirational water loss. Plant World 16:1–35. 1913.

9. LIVINGSTON, B. E., and BROWN, W. H., Relation of the daily march of transpiration to the variation of the water content of foliage leaves. BOT. GAZ. 53:309–330. 1912.
10. LIVINGSTON, B. E., and SHREVE, EDITH B., Improvements in the methods of determining the transpiring power of plant surfaces by hygrometric paper. Plant World 19:287–309. 1916.
11. LLOYD, F. E., The physiology of stomata. Carnegie Inst. Wash. Publ. no. 82. 1908.
12. MARVIN, C. F., Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew point. U.S. Dept. Agric. Weather Bur. Bull. 235. 1912.
13. RENNER, O., Experimentelle Beiträge zur Kenntniss der Wasserbewegung. Flora 103:171–247. 1911.
14. RYSSELBERGHE, F. VAN, Influence de la température sur la permeabilite du protoplasme vivant pour l'eau et les substances dissoutes. Bull. Acad. Belg. 1:173–221. 1901.
15. STAHL, E., Einige Versuche über Transpiration und Assimilation. Bot. Zeit. 52:117–146. 1894.
16. TRELEASE, S. F., and LIVINGSTON, B.E., The daily march of transpiring power as indicated by the porometer and by standardized hygrometric paper. Jour. Ecol. 4:1–14. 1916.

REPEATED ZOOSPORE EMERGENCE IN DICTYUCHUS[1]

WILLIAM H. WESTON

(WITH PLATE XXIII AND ONE FIGURE)

Dictyuchus, one of the less known genera of the Saprolegniaceae, was established in 1869 by LEITGEB (6) to include a single species, *D. monosporus;* and in 1872 LINDSTEDT (7) added the two species *D. polysporus* and *D. Magnusii;* while in 1893 a fourth species, *D. carpophorus*, was described by ZOPF (12). LEITGEB observed that in his type species laterally biciliate zoospores emerged from sporangiospores which were invariably retained *in situ*, and that these zoospores swarmed but once; and since this condition has been found in all the other species, it has been regarded as characteristic of the genus.

In this paper certain observations on an undetermined species of *Dictyuchus* are presented, which show that, in this instance at least, the usual history, as described by LEITGEB and others, may be modified through the presence of a second swarming of laterally biciliate zoospores, which occurs after the first has been completed; and since, so far as the writer is aware, a diplanetic condition of this type has not hitherto been noticed in any of the Saprolegniaceae, a record of its occurrence has seemed desirable, even before a comprehensive study of the fungus has been completed.

The *Dictyuchus* in question appeared in a culture of moist sand, leaves, and other débris taken from a shaded brook bed in a ravine near Great Barrington, Massachusetts; and for over a year and a half it has been kept under observation both in gross and pure cultures, which have been subjected to a variety of cultural conditions. During this period, however, all attempts to induce the formation of sexual organs have been without result, although sporangia were readily and abundantly produced, and it is thus impossible to reach any definite conclusion as to its specific identity. That it cannot be referred to *D. carpophorus* Zopf is evident

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 85.

from the quite different form of its sporangia, as well as from the absence of the peculiar tubercles which, according to ZOPF, are characteristic of that form. On the other hand, it is probable, as has been suggested by VON MINDEN (8) and FISCHER (4), that the sexual organs which LINDSTEDT found associated with his *D. polyspora*, and on which he based the species, belonged in reality to a member of some other genus which had accidentally been introduced into his cultures. For this reason the species is perhaps best regarded as in all probability invalid; and there thus remain but two others, *D. Magnusii* Lindst. and *D. monosporus* Leitg., with which the present form may be compared. On a basis of sporangial characters alone it might readily be referred to either of these species, and its failure to produce sexual organs may be due to the fact that it is the antheridial strain of a unisexual (dioecious) type, similar to that which both of the last mentioned species are said to illustrate. On the other hand, it may prove to be a neutral strain, comparable with PIETERS' (9) "*Saprolegnia* no. 66" and the undetermined species of *Achlya* studied by the author (11), having lost its ability to reproduce sexually, at least under ordinary conditions. That this may be the correct explanation is further suggested by the fact that such neutral or non-sexual conditions of *Dictyuchus* have previously been reported by HUMPHREY (5), TIESENHAUSEN (10), and others; while VON MINDEN even definitely identifies a form of this nature with *D. monosporus* Leitg. Although it is quite possible that a similar disposition of the present species might prove to be the correct one, a definite specific reference does not seem justified at the present time.

In order to follow its development in detail, cultures which were known to be uncontaminated by other forms were washed repeatedly in sterile water and placed in a drop on a slide. As soon as the zoospores had emerged, swarmed, and come to rest, they were picked up with a capillary pipette, placed in a few cubic centimeters of sterile water, and sprayed by means of an atomizer on nutrient media contained in Petri dishes. The latter were then examined under a low magnification, the positions of single isolated spores were marked, and, after two or three days'

growth, transfers to stock cultures were made from such mycelia as proved to be uncontaminated. The development of the fungus thus isolated was studied for over a year in Van Tieghem cells, Petri dishes, and battery jars, under a great variety of cultural conditions.

The mycelium in its morphological characteristics is very similar to that of other species of Saprolegniaceae which have been grown in pure culture. Physiologically, however, it is characterized by weak growth, and in consequence requires more frequent transfer and more concentrated nutriment for successful maintenance. The process of sporangium formation in its early stages closely resembles that which is usually found in other members of the family. In the young sporangium initials, filled with dense protoplasm, hyaline clefts arise, extend, and divide the contents into subequal, polygonal spore initials. The sudden shrinking of the sporangium with a concomitant vacuolation of the spore initials now takes place; but the vacuolate condition is more persistent than in other genera, since one or two vacuoles are often retained in the spores at maturity.

In the succeeding stages of its development, however, the sporangium shows itself to be quite different from any other member of the family, save perhaps that of the doubtful genus *Aplanes*. During the swelling of the individual sporangiospores which marks the final stage of development of the sporangium, it becomes apparent that the delicate membrane surrounding each spore has become firmly united, not only to the walls of the adjacent spores, but also to the inner surface of the sporangial membrane. This close union of the walls appears to be a fundamental peculiarity in sporangia of the generic type, and sharply distinguishes it from the abnormal but superficially similar conditions which are occasionally encountered in *Saprolegnia*, *Achlya*, and *Thraustotheca*, when large numbers of spores have failed for some reason to make their escape. We are probably justified, moreover, in accepting HUMPHREY'S (**5**, p. 81) tentative suggestion that this characteristic obtains throughout the genus. Study of the empty sporangium alone (fig. 1) might lead one to interpret its so-called "cell-net" structure as the result of a simple division

into cells, rather than of a process of progressive cleavage; but continuous observation of the successive stages of spore formation affords no evidence in support of this assumption. Moreover, in starved sporangia only partially filled with protoplasm the spores when formed are more separate, and on swelling do not become closely pressed together. Consequently, only an incomplete union of the walls takes place (fig. 2); and the spores become rounded off to a greater degree, so that the resultant structure is more easily understood than the "cell-net" condition in the densely filled sporangia. It is of interest to note that ZOPF (12, *pl. 3, fig. 11*) also figures a similar condition in *D. carpophorus*. This union of the walls in the sporangium increases the mechanical strength of the structure; and in consequence the final swelling of the spores is resisted, and they do not burst out of the sporangium, even though it becomes swollen and bulged. Frequently, however, the swelling of the spores, combined with the outward bulging of the terminal wall of the sporangiophore, is sufficient to rupture the sporangial wall at the base (fig. 19), and the sporangium is abjointed as a whole. This occurs quite commonly even in vigorous cultures, as VON MINDEN (8) has observed, and does not appear to be the result of degeneration and senescence, as LEITGEB (6) and FISCHER (4) have stated.

Renewal of the sporangia is effected by cymose branching (fig. 2), and by their formation in basipetal succession (fig. 1). The first method has been regarded as characteristic of *D. monosporus*, and the second as typical of *D. Magnusii;* but the regular occurrence of both methods in our form and even in *D. Magnusii*, according to VON MINDEN, would indicate that these characters are not specific.

In their subsequent development the sporangiospores within the indehiscent *Dictyuchus* sporangium may either emit zoospores or give rise to hyphae of germination. Since LEITGEB'S original description of these processes is quite detailed, only additional or significant points need be mentioned here. The emergence of the zoospore from the sporangiospore, although described at length by this author, is only scantily figured. The accompanying drawings (figs. 3–9), therefore, have been made to illustrate this process.

After the zoospores (figs. 8, 9) have emerged, their general structure and the disposition of the cilia are seen to be very similar to the "secondary" laterally biciliate type in other genera of the family, although these zoospores are longer and more tapering than those of *Achlya* and *Thraustotheca*, while more flattened than those of *Saprolegnia*. On an average the zoospores of our form are about 13 μ long and 10 μ wide. After an active period of variable duration they come to rest, lose their cilia, and encyst, forming spherical, coarsely granular spores which, to avoid confusion, will be called "cystospores" (fig. 10). After a time these may germinate by sending out hyphae in a perfectly normal manner (figs. 16, 17).

When the sporangiospores give rise not to zoospores but to hyphae of germination, the latter push through the enveloping sporangium wall and grow out into the water (fig. 18) in a manner quite similar to that which is said to be characteristic of *Aplanes*. It is to be noted, however, that in *Dictyuchus*, as well as in other genera in which it occurs as an abnormal method of development, such germination only takes place in the presence of nutrient substances, or of such non-nutrient materials as prevent zoospore emergence.

It is clear that the cycle of non-sexual spore formation just described agrees entirely with the usual accounts. In the form under discussion, however, an additional zoospore emergence may take place. This phenomenon was repeatedly observed under the following conditions: A piece of agar covered with mycelium, when transferred from a stock culture to dilute beef extract, rapidly grows to a small compact tuft of hyphae. If this tuft, after a thorough washing to remove the adhering nutriment, is placed in a hanging drop for study, sporangium formation rapidly takes place, and from the sporangiospores large numbers of zoospores emerge and swim about. Finally they come to rest and encyst for the most part along the edge of the drop. After a time some of the encysted spores so situated germinate by hyphae. Many, however, emit zoospores, with the result that all along the edge of the drop may be seen empty cystospores and zoospores

in various stages of emergence. This process, as shown in figs. 10–13, closely resembles the previous emergence of the zoospore from the retained sporangiospores. The zoospore emerging from the cystospore moreover is exactly like the original zoospore which emerged from the sporangiospore. The cultures in which this additional emergence of the zoospore was observed were derived from single spores, and their purity was beyond question.

To appreciate the significance of this repeated zoospore emergence in *Dictyuchus*, it is necessary to consider briefly the corresponding phenomena in certain related types. The genera of the Saprolegniaceae, as is well known, are distinguished by the characteristic peculiarities of their non-sexual reproduction, since a regular and distinct cycle of non-sexual spore production marks each separate genus. It has been customary to arrange the genera in a series in accordance with the degree of simplicity or the complexity of their cycles. Disregarding the question whether such a series represents the elaboration of a simple type or the simplification of a complex one, we may first consider *Saprolegnia*, which has the most extensive cycle. Since in the other main genera the cycles are less extensive in increasing degrees, we may, in accordance with this, arrange a convenient series: *Saprolegnia*, *Achlya*, *Thraustotheca*, *Dictyuchus*, and *Aplanes* (text fig. 1).

The characteristic cycles of non-sexual reproduction in these 5 genera are shown in the accompanying diagrams, which follow the accepted descriptions for all but *Thraustotheca*. In this genus the author has found that the non-motile sporangiospores swell and escape by bursting the enveloping sporangium wall, after which they closely resemble the escaped sporangiospores of *Achlya* in their further development. The genera form a series representing a gradual decrease in the extent of the cycle of non-sexual spore formation. The series ranges from *Saprolegnia*, with its swarming of primary and secondary zoospores, through successive stages to *Aplanes*, which is believed to lack both of these phases of zoospore activity.

In the repeated emergence of its zoospores this species of *Dictyuchus* shows a distinct departure from the cycle of spore formation

that is customarily ascribed to the genus, nor has this phenomenon been reported in the case of other members of the family. In *Pythium*, however, which is more or less closely connected with the Saprolegniaceae, according as one or another of the several theories of relationship is accepted, a similar double swarming of laterally biciliate zoospores has been reported. CORNU (3) in 1872 first noted the fact as follows: "les zoospores dans tous ces genres

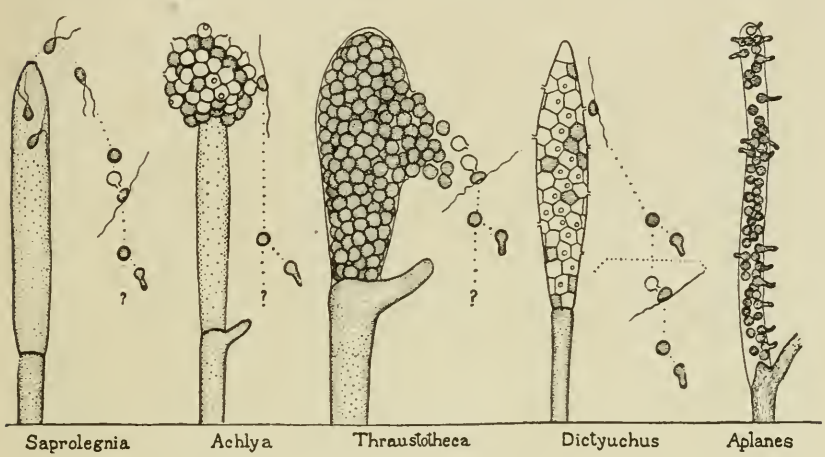


FIG. 1.—Comparative view of cycles of non-sexual spore formation in main genera of Saprolegniaceae; diagrams, of approximately same scale, from drawings of living material, except in case of *Aplanes*, which is after DEBARY'S figure.

germent en donnant lieu à un filament ... ou bien elles émittent des zoospores semblables à elles-mêmes (ex. *Pythium proliferum* et ses var.)." Recently BUTLER (2) has corroborated CORNU'S observations by describing and figuring the process in the case of *Pythium diacarpum*. In spite of the apparent rarity of this phenomenon, however, the writer ventures the opinion that further investigations will bring to light other cases, not only in *Dictyuchus*, but also in related genera of the Saprolegniaceae.

The significance of this repeated zoospore emergence in *Dictyuchus* is a matter of some interest. One may, of course, regard it as a regular but hitherto unobserved stage of the life cycles of

the genus; but in view of the detailed investigations of LEITGEB and others it seems doubtful if such is the case. It must be admitted, however, that *Saprolegnia* had been studied for many years before LEITGEB observed in it the diplanetism on which he based his genus "*Diplanes*." On the other hand, the repeated zoospore emergence in the present instance might be regarded as peculiar to this particular and perhaps hitherto unnoticed species of *Dictyuchus*. Since such an assumption is contrary to our customary conception of the fixity of generic characteristics, its truth may well be doubted. In view of the tardy recognition of the extent of this phenomenon in the genus *Pythium*, it is possible that it has merely been overlooked in the recognized species of *Dictyuchus*. Finally, it is possible that this phenomenon occurs, not only in various species of *Dictyuchus*, but also in other genera of the Saprolegniaceae under certain favorable conditions; and that these conditions either have not been attained in cultures heretofore, or the emergence has escaped observation. It seems highly probable that this is the case, and that in certain Saprolegniaceae there inheres in the protoplasm, even of encysted zoospores of the second type, the ability to form not only germ tubes but also zoospores; and that under proper circumstances the latter may be produced. Probably, then, as ATKINSON (1) has suggested in the case of *Pythium*, this repeated zoospore emergence may best be regarded as a phenomenon of germination, and one which the author believes can be brought about by certain favorable conditions. It is possible that extensive cultural studies of various Saprolegniaceae, with this end in view, will demonstrate that its occurrence is far more widespread than has been suspected. The writer is obliged to admit, however, that he has been unsuccessful in many attempts to induce repeated zoospore emergence in *Achlya* and *Thraustotheca;* but the well known sensitiveness of the Saprolegniaceae to surrounding conditions makes it possible that these failures may have been the result of faulty methods.

In any case, the occurrence of this phenomenon in even a single species of *Dictyuchus* points to the conclusion that the customary application of such terms as "monoplanetic" and "di-

planetic" may be somewhat misleading, and necessitates a modification of our conception of the condition of monoplanetism and diplanetism in the Saprolegniaceae.

Summary

1. In the characteristics of its non-sexual reproduction the fungus which is the subject of this paper shows itself to be a member of the genus *Dictyuchus*. No sexual reproduction was observed, however; hence it cannot be assigned to any of the recognized species.

2. During the formation of spores within the sporangium, the walls of adjacent spores unite with one another and with the enveloping sporangium membrane, to form a polygonally chambered, indehiscent structure. In this respect *Dictyuchus* differs fundamentally from all other Saprolegniaceae, save perhaps the doubtful genus *Aplanes*.

3. The zoospores which emerge from the sporangiospores come to rest and encyst as is customarily described. From these encysted spores in turn, however, laterally biciliate zoospores may emerge. This repeated emergence of laterally biciliate zoospores has not previously been reported in any member of the Saprolegniaceae.

4. It is the opinion of the writer that future study will prove that this phenomenon may occur in other species of *Dictyuchus*, and perhaps even in other members of the family.

In conclusion the writer wishes to express his thanks to Dr. Roland Thaxter, under whose kindly supervision these observations were made.

Laboratory of Cryptogamic Botany
Harvard University
Cambridge, Mass.

LITERATURE CITED

1. Atkinson, G. F., Some problems in the evolution of the lower fungi. Ann. Mycol. 7:441–472. *figs. 20.* 1909.
2. Butler, E. J., An account of the genus *Pythium* and some Chytridiaceae. Mem. Dept. Agric. India, Bot. Series 1:1–160. *pls. 1–10.* 1907.
3. Cornu, M., Monographie des Saprolegniees. Ann. Sci. Nat. V. 15:5–198. *pls. 1–7.* 1872.

4. FISCHER, A., Saprolegniineae, in Rabenhorst's Kryptogamenflora, 1^4:310–383. *figs. 13.* 1892.
5. HUMPHREY, J. E., The Saprolegniaceae of the United States. Trans. Amer. Phil. Soc. 17:1–148. *pls. 7.* 1893.
6. LEITGEB, H., Neue Saprolegnieen. Prings. Jahrb. Wiss. Bot. 7:357–387. *pls. 22–24.* 1869.
7. LINDSTEDT, K., Synopsis der Saprolegnniaceen und Beobachtungen über einiger Arten. Berlin. pp. 69. *pl. 4.* 1872.
8. MINDEN, M. VON, Saprolegniineae, in Kryptogamenflora der Mark Brandenburg, 5^4:209–600. 1912.
9. PIETERS, A. J., New species of *Achlya* and *Saprolegnia*. BOT. GAZ. 60: 483–490. *pl. 1.* 1915.
10. TIESENHAUSEN, M., Beiträge zur Kenntnis der Wasserpilze der Schweiz. Arch. Hydrobiol. Planktonk., Biol. Sta. Plön. 7^2:261–308. 1912.
11. WESTON, W. H., Observations on an *Achlya* lacking sexual reproduction. Amer. Jour. Bot. 4:354–367. *pl. 18.* 1917.
12. ZOPF, W., Über eine Saprolegniee mit einer Art von Erysipheenähnlicher Fruchtbildung. Beiträge Physiol. Morphol. Nied. Org. 3:48–59. *pls. 2, 3.* 1893.

EXPLANATION OF PLATE XXIII

The figures were drawn from living material at the level of the stage with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been reduced to about two-thirds of their diameter in reproduction.

FIG. 1.—Two successively formed sporangia, the terminal empty, showing "cell-net" structure; second still containing spores; ×550.

FIG. 2.—Sporangium developed from starved hypha, showing incomplete uniting of spore walls; ×550.

FIGS. 3–7.—Stages in emergence of zoospore from sporangiospore; ×1400.

FIG. 8.—Zoospore after liberation; oblique later view; ×1400.

FIG. 9.—Same just starting to swim away; side view; ×1400.

FIG. 10.—Encysted zoospore (cystospore); ×1400.

FIGS. 11–13.—Stages in emergence of zoospore from cystospore; ×1400.

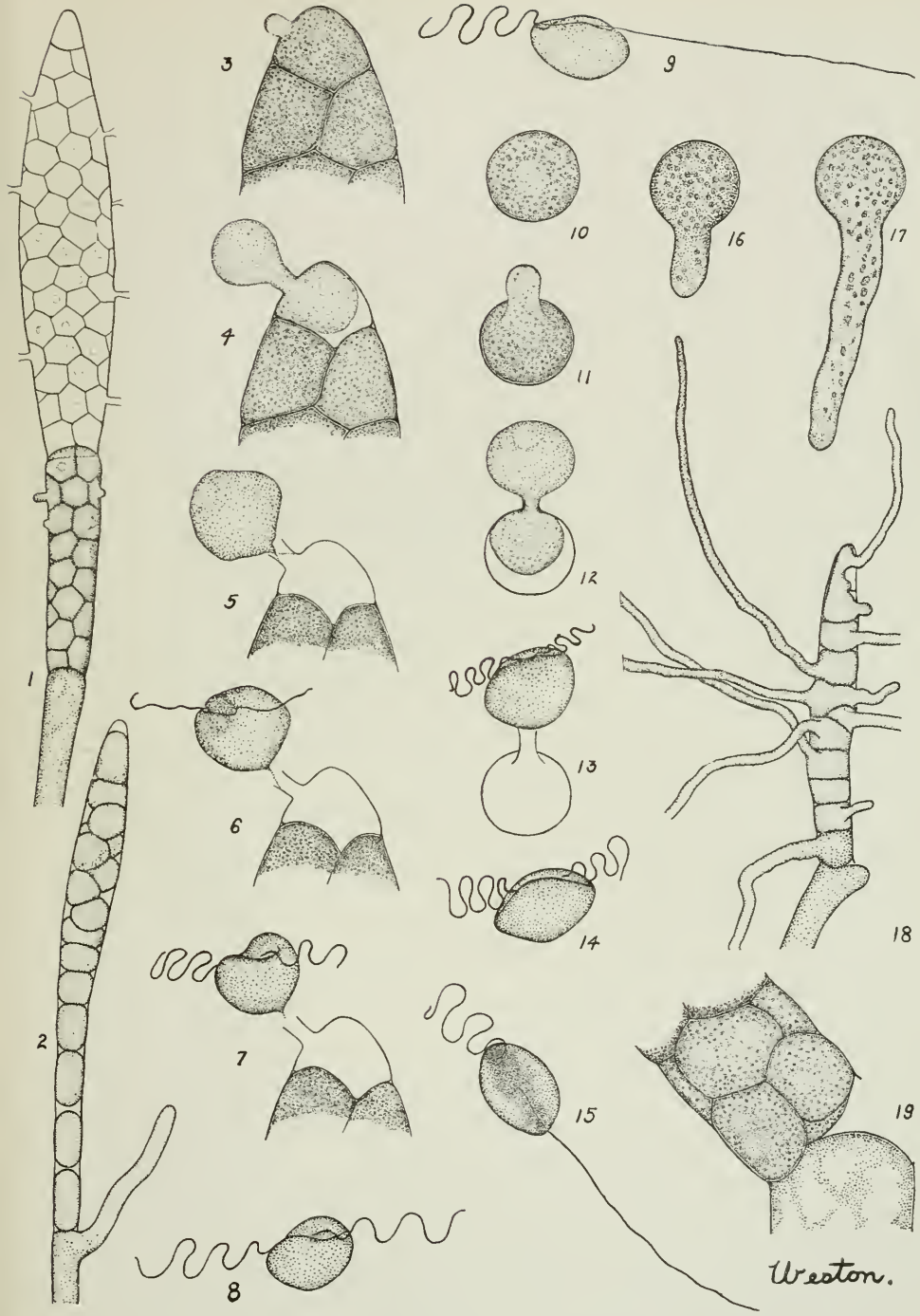
FIG. 14.—Zoospore just after emerging; side view; ×1400.

FIG. 15.—Same just starting to swim away; looking down on grooved surface; ×1400.

FIGS. 16–17.—Cystospore germinating by formation of hypha; ×1400.

FIG. 18.—Germination of sporangiospores *in situ* by hyphae; ×550.

FIG. 19.—Separation of sporangium from its hypha by rupture of sporangium wall at base; ×1400.



WESTON on DICTYUCHUS

RELATION OF NUTRIENT SOLUTION TO COMPOSITION AND REACTION OF CELL SAP OF BARLEY

D. R. Hoagland

In recent years considerable attention has been given to the cell sap from different plants, especially as influenced by varying soil and climatic conditions. Some very interesting general relations have been brought out, but in these experiments it has not been possible to ascertain or control the exact concentration and composition of the soil solution. McCool and Millar (4), however, have made numerous measurements of the freezing point depressions of the cell sap of both tops and roots of plants growing in soils and nutrient solutions of varying osmotic pressure. These researches have shown clearly that the sap of the plant, particularly of the roots, reflects the concentration of the nutrient solution, whether in the soil or in water cultures.

Comparatively few measurements of the conductivity of the cell sap have been made, although some data concerning this point are quoted by Atkins (1). The H ion concentration and chemical analysis of the sap have received still less study, yet all these determinations are of the greatest importance in soil fertility investigations. The total osmotic pressure in the plant is known to be dependent to a considerable extent on intensity of photosynthetic action, as well as on the nutrient solution, while the inorganic constituents may well have a more direct relation to the surrounding media.

For a number of years this laboratory has been engaged in the investigation of the relation between the growth of the barley plant and the composition and concentration of the soil solution as shown by analyses of water extracts and freezing point determinations by the method of Bouyoucos and McCool (2). The work with soils has made it evident that the soil solution is of paramount importance in its effect on crop growth, while, on the other hand, the plant has a marked influence on the concentration and composition of the soil solution. It soon became apparent that the

elucidation of these difficult relationships would require further study by the methods of water and sand culture, which may be subjected to more rigorous control. It is thought that some of the data pertaining to the cell sap of plants from these various soil, water, and sand culture experiments are worthy of a brief discussion at this time.

The soils were kept in large tanks under controlled conditions as described by STEWART (6). The technique of the water and sand cultures will be described elsewhere. The procedure was designed to place, so far as possible, no limitation on the growth of the plant other than the variables under investigation. The nutrient solutions were made to have a composition similar to that of the soil extracts with respect to the important elements. Various concentrations of both acid and neutral reaction were employed. In each concentration acid and neutral solutions had an almost identical osmotic pressure, and the relation between the various ions was very similar. The reactions were governed by the hydrolysis of the various potassium phosphates used.

The procedure employed in obtaining the cell sap consisted in cutting the plant into small pieces, freezing first in brine, then in a carbon dioxide ether bath, and finally pressing out the sap as thoroughly as possible through cheesecloth. It is realized that the exact concentration of the sap is dependent upon the technique employed, but the results are comparative and the general magnitudes, which are of interest in this discussion, are probably not far different from those obtainable with other methods of extraction. Osmotic pressures were determined by the freezing point method, conductivity measurements in the usual manner at a temperature of 25° C. The hydrogen ion concentrations were measured with the aid of the hydrogen electrode, using the apparatus described by SHARP and the author (5). The data for the freezing point depressions, conductivity, and hydrogen ion concentration of the various cultures are summarized in table I.

Effect on osmotic pressures

Considering the total osmotic pressures first, it will be noted that both the tops and roots, either in the acid or neutral solutions, reflect the concentration of the nutrient solution, although no very

definite relationship is apparent. The highest osmotic pressure is found in the sap from the plants grown in the acid nutrient solution of highest concentration (1.72 atmospheres). The roots of these plants showed marked evidence of injury. In all cases

TABLE I

OSMOTIC PRESSURE, CONDUCTIVITY, AND H ION CONCENTRATION OF PLANT SAP IN WATER, SAND, AND SOIL CULTURES

Total solids approximate p.p.m.	Nutrient solution			Plant sap from tops			Plant sap from roots		
	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H
	Barley plants 8 weeks, grown in water cultures								
200 ...	0.07	3870	5.50	8.01	97	5.68	3.62	125	6.44
200 ...	0.07	3230	6.53	7.55	76	5.68	3.69	137	6.12
1400 ...	0.58	550	5.48	8.13	61	5.85	4.11	117	6.83
1300 ...	0.56	528	6.83	8.35	66	5.82	.....	.....	.....
2300 ...	0.88	347	5.14	8.86	65	5.99	5.04	102	6.97
2200 ...	0.88	347	6.76	10.26	50	5.90	4.52	119	7.08
4500 ...	1.72	196	4.94	11.40	53	5.95	4.90	95	.....
4300 ...	1.70	189	6.14	10.24	53	5.90	5.63	77	6.97
	Barley plants 7 weeks, grown in sand cultures								
2500 ...	0.94	335	6 97	10.66	46.9	6.12			
5000 ...	1.81	220	6.97	11.74	42.0	6.15			
8000 ...	2.75	148	6.97	12.24	38.9	6.20			
	Barley plants 6–7 weeks, grown in 6 different soils								
600 ...	0.22*	.....	7.03	9.96	59.9	0†			
600 ...	0.21	.....	7.15	9.52	60.4	6.12			
900 ...	0.33	.....	7.15	10.96	64.2	6.15			
500 ...	0.16	.....	7.03	10.14	69.2	0†			
500 ...	0.19	.....	7.17	9.97	71.5	5.99			
200 ...	0.08	.....	7.34	9.11	65.8	6.12			

* This column represents concentration of soil solution at about time plants were collected; previously the concentration was higher.

† No determination was made.

the osmotic pressure of the tops is much greater than that of the corresponding roots. These findings are in general agreement with those of McCOOL and MILLAR. The tops of the plants grown in the various soils have osmotic pressures similar to those found in the water culture experiments. The osmotic pressures of the soil solutions, as determined by the method of BOUYOUCOS and McCOOL, varied between 0.08 and 0.33 atmospheres at

about this stage of growth of the plant. They are thus quite comparable with the concentrations of certain of the solutions used in the sand and water culture experiments. In every instance the osmotic pressure of the plant sap is much higher than that of the nutrient medium.

All of the samples of plant sap have a very high specific conductivity, and very decided variations are exhibited by the different water cultures. The concentration of the nutrient solution has unquestionably had a pronounced effect on the electrolyte content of the cell sap. A somewhat greater resistance is found in the root sap than in that from the tops. It is to be noted, however, that the variations in conductivity due to the nutrient solution are generally as great for the tops as for the roots, but in either case only a general relationship is apparent. The conductivities of the plant saps from the various soils were very similar in magnitude to those of the plants grown in water culture solutions of 0.5 to 0.9 atmospheres osmotic pressure. The conductivities of the sand culture plants were somewhat greater, but these plants were two weeks younger. In each case the expressed cell sap has a much higher concentration of electrolytes than the surrounding media, the relationship being 50:1 in the case of the most dilute solution. Thus the nutrient media, the root sap, and sap from the tops show three very dissimilar levels of electrolyte concentration. That the simple laws of diffusion are not sufficient to explain the equilibria involved in the plant absorption and metabolism is well recognized, and the data now presented strikingly illustrate this point of view, with reference to the ion content of the plant and its nutrient solution.

H ion concentration of sap

HAAS (3) and TRUOG (7) have shown that the sap of plants quite generally has an H ion concentration distinctly on the acid side. Determinations of H ion concentration were made on the samples of sap obtained as previously described. Table I shows a comparison of the acidity of the sap from plants grown in water cultures of very different concentration and reactions, as well as in sand and soil media.

It is evident that all samples of tops have almost the same P_H value, although the electrical resistances and osmotic pressures may vary widely. It would seem that the reaction is governed by a definite buffer system. In this connection it will be of interest to state that other experiments reported elsewhere have shown that the plant possesses a marked regulatory influence in the selective absorption from the various phosphoric acid anions; that is, either an alkaline or acid nutrient solution has its reaction quickly changed to approximate neutrality.[1]

The measurements of H ion concentrations on the sap from the tops were very definite and constant, but the determinations on the root sap were less satisfactory. An increase in alkalinity was noted during the measurement, possibly due to the reduction of NO_3 and the absence of a sufficient buffer effect. Apparently, however, the sap expressed from the roots has a nearly neutral reaction in several cultures.

Chemical analyses of plant sap

The analyses presented in table II were made on the expressed sap from plants grown in 6 soils of different origin and productivity. The soils were kept at optimum moisture content and under strictly controlled conditions, and 6 or 7 weeks after planting one or two tillers were separated from each of about 20 plants for each soil examined. The sap was obtained by the procedure already described, and then diluted and filtered through a porcelain candle to separate out any suspended material. The analyses were made by the methods described by STEWART (6).

The content of the individual ions substantiates the high conductivity measurements on the sap. All ions are present in relatively great concentration, including the NO_3 ion. Some idea of the relation between the composition of the cell sap and soil solution may be gained by comparison with the data for the soil extracts made at about the same time. There is reason to believe that the relation of several important ions is somewhat

[1] Later experiments have indicated that the HCO_3 ion formed is of greatest importance in regulating the reaction of complete nutrient solutions. A solution of KH_2PO_4 alone retains about the same P_H value.

similar in the soil solution and soil extract. As previously shown, the extract may contain several times the quantity of solutes actually found in the soil solution; that is, in the free water of the soil when it contains optimum percentage of water. The magnitudes have been calculated by the methods of BOUYOUCOS and MCCOOL. Estimates of the concentration of ions present in the soil solution indicate that invariably the plant sap has a much greater concentration, and in the case of K and PO_4 ions many times greater. It is evident that the relation of the ions to each other is also quite different in the two cases. In the soil solution

TABLE II

ANALYSES OF PLANT SAP AND SOIL EXTRACTS

SOIL NO. AND TEXTURE	P.P.M. OF EXPRESSED SAP					P.P.M. OF 1:5 SOIL EXTRACT†				
	Total nitrogen*	Ca	K	Mg	PO_4	NO_3	Ca	K	Mg	PO_4
Silty clay loam 3.....	864	680	5540	300	840	90	70	50	20	9
Silty clay loam 5.....	1030	640	5660	280	1360	70	90	30	20	21
Fine sandy loam 8....	1370	920	6130	280	920	60	70	30	20	12
Fine sandy loam 9....	820	1040	5240	290	870	30	30	25	10	10
Fine sandy loam 11...	680	620	5690	280	1960	50	40	50	15	30
Fine sandy loam 12...	940	640	5440	340	1420	30	30	30	10	12

* From 30–50 per cent in form of NO_3 nitrogen.

† Extracts are much more dilute than soil solution, the total concentration of which is shown by table I.

Ca is present in about the same magnitude as K; in the plant sap the concentration of K is from 5 to 10 times that of Ca. The ratio of Ca to Mg is not very dissimilar, Ca exceeding Mg in all samples, both in the plant and soil.

The question has often been discussed whether the plant reflects the composition of the soil. It is the general consensus of opinion that the analysis of the plant ordinarily gives no indication of the deficiencies or fertilizer needs of a soil. This could scarcely be expected, of course, in view of the complex and changing nature of the two systems of the plant and soil. Moreover, analyses of the mature and ripened plant, such as usually have been made, could not possibly give any insight into the relation of the plant to the soil in the period of active growth. It cannot be too strongly emphasized that both the plant and soil are

dynamic systems. The soil solution, as previous work in this laboratory has definitely shown, is changing from day to day, and gradually attains a very low concentration at the time when the plant has completed its maximum absorption. Without consideration of these phenomena it would be useless to hope for any clear understanding of soil fertility problems as related to plant requirements.

That some relation must exist between the inorganic elements in the plant and its nutrient solution is apparent from the data obtained in the water culture experiments. Similarly, concentration and composition of the soil solution should affect the absorption by the plant, but here it is very difficult to establish the relationship. For this purpose it would be necessary to appraise not only the concentration of the soil solution at a given time, but its potentiality for renewal. In the work previously referred to an idea of this factor has been obtained by comparing cropped and uncropped soils. The use of this method has made it possible to show some rather definite relations between water extracts of the soils and crop yield, but in the experiment now under consideration the composition of the sap at the given period bears no constant relation to the final yield of the crop, nor to the composition of the water extracts. An exception to this statement may possibly be found in the case of phosphorus, where the concentration of PO_4 in the soil solution is not improbably reflected in the sap. The K and Mg, and to a less extent the Ca, are of approximately the same magnitudes in all samples. While the soils in question varied considerably in their productivity, apparently the average concentrations of the soil solutions were not sufficiently different clearly to influence the concentration of the cell sap.

It does not follow from the foregoing that further study will not indicate a connection between the soil solution and the elements absorbed by the plant. Before the question is decided it will be necessary to examine not only the cell sap, but the total composition and yield, with the strictest control of the soil and study, not of the ripened plant, but the plant in the various stages of active metabolism.

Summary

The expressed sap from barley plants grown in water, sand, and soil cultures under controlled conditions has been examined with the following results:

1. The osmotic pressures in the sand and water cultures are reflected in the cell sap of the tops and roots.

2. The electrical conductivity of the nutrient solution has a marked influence on the conductivity of the sap. This is as marked for the tops as for the roots. The conductivity of the plant sap is from 4 to 50 times greater than that of the nutrient solution.

3. The sap from the tops of all plants grown in sand and soil cultures or water cultures of different concentrations and reactions had almost the same P_H value, approximately 6.0.

4. Samples of sap from plants grown on 6 different soils under the same climatic conditions were analyzed for important elements. In every case the concentration in the sap was found to be very much greater than in the soil solution.

5. The dynamic nature of the relation between the soil solution and the plant is emphasized.

Division of Agricultural Chemistry
California Agricultural Experiment Station
Berkeley, Cal.

LITERATURE CITED

1. Atkins, W. R. G., Recent researches in plant physiology. London. 1916.
2. Bouyoucos, G. J., and McCool, M. M., The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agric. Exp. Sta. Technical Bull. no. 24. pp. 1–44. 1915.
3. Haas, A. R., The acidity of plant cells as shown in natural indicators. Jour. Biol. Chem. **27**:233–241. 1916.
4. McCool, M. M., and Millar, C. E., The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. Soil Science **3**:113–138. 1917.
5. Sharp, L. T., and Hoagland, D. R., Acidity and absorption as measured by the hydrogen electrode. Jour. Agric. Research **7**:123–145. 1916.
6. Stewart, G. R., Effect of season and crop growth in modifying the soil extract. Jour. Agric. Research **12**:311–368. 1918.
7. Truog, E., Soil acidity. Its relation to the growth of plants. Soil Science **5**:169–195. 1918.

BRIEFER ARTICLES

PARAFFIN SOLVENTS IN HISTOLOGICAL WORK

In the last few years there have been described in the BOTANICAL GAZETTE two ways of improving the common method of replacing xylol with paraffin in histological work. The alleged defect in the old method lies in the fact that the paraffin sinks to the bottom of the vessel and immediately surrounds the specimens with a concentrated solution. LAND'S[1] remedy for this is to support the paraffin near the surface of the xylol in a basket of wire gauze; GOODSPEED[2] molds the paraffin into a lump that will fit the containing vessel and rest on top of the xylol. Both of these methods have been found good for accomplishing the end desired, and a third may be of interest.

The paraffin to be used can, by aëration, be rendered capable of floating in xylol, the method of preparation being similar to that employed in giving buoyancy to some kinds of soap. While the paraffin is at a temperature only a degree or two above that required for melting, a current of cold air is bubbled through it, causing it to harden as a frothy mass. This mass is then kneaded to secure finer grain and more even distribution of the air bubbles. Only a small amount of air is needed to produce the proper buoyancy, and it can be supplied from any one of numerous sources and cooled by passing through a condenser. Although the method may seem troublesome, it is really not so; a large amount of paraffin may be prepared at one time, and nothing further is necessary to get the desired results.

Although this method of holding the paraffin at the top of the xylol has been found successful and easy of manipulation, I do not employ it as a rule, because I have not yet seen the defect of the old method of adding the paraffin when xylol is used, and chloroform has been found perfectly satisfactory in all cases. If, when xylol is used, the necessary amount of paraffin be added in a finely divided condition in 5 or 6 portions, and diffusion aided by gentle agitation, a

[1] LAND, W. J. G., Microchemical methods, an improved method of replacing the paraffin solvent with paraffin. BOT. GAZ. 59:397. 1915.

[2] GOODSPEED, T. H., Method of replacing paraffin solvent with paraffin. BOT. GAZ. 66:381–382. 1918.

sufficiently gradual change is secured. When the paraffin is to be supported at the surface of the solvent, by buoyancy or other means, it is better to add it in a round lump, because in this case slow diffusion is desirable; but, when it is allowed to sink in the xylol, it should be added in small fragments, since quick diffusion is desirable.

Although in many laboratories xylol has taken the place of chloroform in work of this kind, the latter is still used by many technicians because of certain advantages that it possesses over xylol. The object in using either is not, as is often stated, to "clear" the specimen, but to provide a medium for the introduction of the paraffin. Actual "clearing," that is, the production of transparency, is of no benefit at all in this stage of the process; in fact, it proves a disadvantage that can be overcome only by staining *in toto* when only a few sections from a long ribbon are to be selected for mounting. Chloroform has much less tendency to produce transparency than has xylol. Moreover, as DUGGAR[3] has stated, a peculiarly undesirable optical effect is produced permanently by xylol in some tissues. The use of chloroform makes unnecessary, of course, any device for holding the paraffin at the top, if this is thought necessary.

The higher specific density of chloroform, which LAND[4] has mentioned as a factor to be considered, is aside from the question; it has long been known that permeability and osmotic pressure, the things which determine shrinkage, are not, as was once supposed, determined by density when pure substances are being compared. Moreover, neither chloroform nor xylol could "plasmolyze" a cell, as LAND states; at this stage of the process a cell is not subject to such life phenomena as plasmolysis.

The item of expense, which has caused the use of chloroform to be discontinued in many places, and which is of special significance just now, can be lessened by the easy and inexpensive recovery of about one-half of the reagent used in the ordinary process. The waste chloroform, containing alcohol or other impurities, is thrown out of solution with a large quantity of water, dried with calcium chloride, which removes both alcohol and water, and distilled, the boiling point being observed as a check on the purity of the product.—PAUL WEATHERWAX, *Indiana University.*

[3] DUGGAR, B. M., Fungous diseases of plants. New York. 1909. p. 49.

[4] In a note added to an article by MOTTIER, BOT. GAZ. **61**:253. 1916.

CURRENT LITERATURE

BOOK REVIEWS

Practical botany

A widespread belief that botany should be so taught and adapted that it should be of service in its industrial applications, particularly agriculture, has led to the publication of several texts with this in view. The production of such a text demands an author who is not only trained in botany, but also familiar with the practical bearings of the subject. The two books reviewed here were written to meet the real or supposed need indicated.

Cook[1] states that he has aimed at three things: "(1) a brief statement of the recognized facts and principles concerning plants and plant growth usually given in textbooks for secondary schools; (2) a list of simple exercises and suggestions for observations which the pupil can conduct without great difficulty and which will demonstrate many of the statements given in the book; (3) a list of questions which are intended to be suggestive to the pupils and to encourage further studies." "The title, 'Applied economic botany,' implies (1) that it is intended as a guide to experimental work in the study of plants, such as should be carried on in any high school, and (2) that it is intended as a preliminary work to the agricultural studies which are now recognized in many high schools."

The first part treats of seeds, roots, stems, buds, leaves, flowers, reproduction, fruits, anatomy, chemical composition, food, and growth, and also outlines the great groups. In short, it is in general the usual botanical foundation given in secondary schools. There are also chapters dealing with forestry, plant diseases, plant breeding, and weeds. Much space is given to "exercises" and "questions," since it is through these that the author aims to lead the student to nature for facts, holding that "we cannot, in the short time allowed to the subject, expect to learn much about many thousands of plants . . . . but we can learn certain principles of plant growth . . . . to be close observers . . . . to be accurate in our experiments and in making records." The exercises are so selected that the material is easily available, for they are based on plants familiar to all and usually of economic importance.

The second part treats of families, discussing the chief economic representatives of each, for example, celery, parsnip, carrot, etc., among the Umbelliferae, and coffee and quinine among the Rubiaceae. The general characters of

[1] Cook, M. T., Applied economic botany. 8vo. pp. xviii+261. *figs. 142.* Philadelphia: J. B. Lippincott. 1919.

the family are given concisely, as well as the more interesting features of the history, distribution, and uses of the more important economic representatives.

The book is written in attractive style, and the material is well selected, and is a commendable effort to differentiate secondary-school botany from university botany. The numerous half-tones are of unusually good quality.

MARTIN[2] has attempted the same task, except that his book is more specifically directed to the botanical needs of the student of agriculture. The first part deals with flowers, seeds, cells, roots, stems, buds, leaves, etc. The application to agriculture consists chiefly in the fact that economic plants are used as illustrative material. For example, oats, corn, wheat, pineapple, tomato, etc., are made to show the usual fundamental facts of morphology. The second part presents an outline of the plant kingdom, from Thallophytes to Angiosperms, along with chapters on ecology, evolution, heredity, and plant breeding.

The presentation throughout is botanical rather than agricultural, a foundation for agricultural study rather than a study of agriculture. The line drawings are not as well done or as accurate as they should be, and the illustrations in general are in contrast with the excellent presswork and the easy and pleasing style of presentation.—F. L. STEVENS.

NOTES FOR STUDENTS

Physiology of dormancy.—Recent work by CROCKER and HARRINGTON[3] materially increases our knowledge of the physiology of dormancy and germination of seeds, and throws much light on the problems of vitality and respiration. Differences in the optimum temperature requirements for germination of Johnson grass and Sudan grass led to a study of their physiological differences, and a comparison of their behavior with seeds of widely separated groups of plants. The discussion therefore is a general contribution of much significance to seed physiology. The study centers in the relation of catalase content to dormancy and vitality. Some improvements in methods of measuring catalase activity are suggested, chief of which is neutralization of the hydrogen peroxide used, as it is found to be injuriously acid if unneutralized. Degree of pulverization must be considered, as seeds vary somewhat as to optimum degree of fineness. Bolting cloth of 70–100 mesh gave the best results in the seeds used. Material must also be freshly ground, as degeneration of catalase is rapid after destruction of morphological integrity.

Catalase activity is found to be 28 or 29 times as great in the embryo of Stoner wheat as in the endosperm. Sudan grass and a hybrid between Tunis grass and sorghum gave similar results. Bracts surrounding the caryopses,

[2] MARTIN, J. N., Botany for agricultural students. 8vo. pp. xv+585. *figs. 488*. New York: John Wiley & Sons. 1919.

[3] CROCKER, WM., and HARRINGTON, G. T., Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour Agric. Research 15:137–174. 1918.

however, and sterile florets show very low activity, especially after a year of dry storage. It is suggested that in these cases, where presumably dead tissues give catalase reaction, the activity is a residuum of previous physiological activity. In the grasses, catalase activity decreases continuously from the time the seeds are harvested. This decrease, however, is in no way connected with loss of vitality. Even when the activity has fallen to one-half or one-third of the fresh harvested material, there may still be practically complete vitality, and but slight loss in vigor. The investigation indicates that under certain conditions catalase activity might be used to estimate the age of seeds. It would only be necessary to know the normal rate of activity decrease, to be sure that no accidental destruction of catalase activity had occurred, and to use proper controls of materials of known age and of equal maturity with the seed to be tested.

When dormancy occurs in grasses it is usually caused by seed coat characters. Thorough drying at about 20° C. is the best after-ripening condition, but during drying catalase activity is falling rapidly. Seeds of *Amaranthus retroflexus* also have a dry after-ripening period, but in these the catalase is time-stable, and very little decrease in catalase activity occurs during drying. In contrast to these there are other seeds, as peach, linden, and hawthorn, where dormancy is due to embryo conditions, in which after-ripening, which depends mostly on cool temperature and moisture, is accompanied by a rapid increase in catalase activity and other fundamental time-requiring chemical changes. Such chemical changes, aside from catalase decrease, do not occur in the grasses. Heating air-dry seeds finally reduces both vitality and catalase activity, but the denaturing of the catalase and the proteins upon which viability depends does not run parallel. Catalase decrease commences at once; but viability may increase temporarily, and then decrease to zero before the catalase is destroyed. In other cases viability is soon lost, but catalase activity remains high, as in *Amaranthus*.

Seeds of Johnson grass kept in a germinator at room temperature undergo secondary dormancy and lose their catalase activity rapidly. The respiratory activity falls correspondingly. If these conditions are repeated in seeds buried in the soil, they may have an important bearing on the longevity of buried seeds. If death depended upon destruction of food supply by respiration, decreased respiration would provide longevity. When seeds of Johnson grass germinate, there is a very rapid increase again in the catalase activity. At every point it seems that catalase activity and respiration intensity run parallel. Some tests of oxidase activity were made with the same seeds, and it is shown that oxidase activity decreases with age. Little relation was found between oxidase and after-ripening changes in grasses or peach seeds, and oxidase activity showed no increase in germination, but in those non-living parts, bracts and scales, where catalase activity was low, oxidase activity was relatively high. The oxidase of Johnson grass and the Tunis grass-sorghum hybrid is only slightly sensitive to mercuric chloride poisoning.

Another paper by the same authors[4] describes some experiments made to test the effects of desiccation on vitality of seeds. The seeds of grains and grasses will withstand drying to less than 1 per cent without material loss in germination. Blue grass and Johnson grass can even be dried to 0.1 per cent of moisture without loss in germination, but vigor is greatly reduced in the blue grass. Still further loss of vigor occurred in the blue grass when dried in vacuo at 100° C. for 6 hours, but the germination percentage was not materially reduced. These results negative the statements of EWART that excessive drying changes dormant protoplasm to such an extent that the essential molecular groupings cannot be re-established under conditions for germination.—C. A. SHULL.

Curing timber.—A method of drying timber more uniformly to avoid cracks and shakes in the logs is proposed by STONE.[5] The method is based upon assumptions as to the natural movement of sap in trees which will not meet with favor among plant physiologists. He considers that the water is held in the saturated tracheal walls, evaporates from these walls into continuous vapor-filled lumina, and moves upward through the tubes in response to a partial vacuum produced above by transpiration. Indeed, the water is supposed to travel upward mostly by night, because at that time the leaves are much cooler than the trunk, and would condense the vapor from the tubes, thus filling the cells as reservoirs against the next day's transpiration. Salts are imagined to travel through the cell walls of the tracheae rather than in the transpiration stream, which is nonexistent in STONE'S assumption. It is hard to imagine a conception much more at variance with experimental results of physiological studies.

The actual drying plant suggested is a closed shed, arranged with a cooler at one end, the purpose of which is to condense the moisture as it leaves the logs, in the form of hoar frost, on the principle of the dew pond. Thus the air of the shed will be kept continually dry, and cold dry air constantly circulating through and around the porous logs. He asserts that this would dry each annual layer simultaneously, and that the shrinkage would be regular and occur without cracking. Whether the proposed plant would really result in the uniform curing of timber the reviewer must leave to the practical forester. Perhaps the suggestion is much sounder on the practical side than the assumptions on which it is based would seem to indicate.—C. A. SHULL.

Philippine plant diseases.—REINKING[6] has published an excellent and very useful account of the economic plant diseases of the Philippines, which

---

[4] HARRINGTON, G. T., and CROCKER, WM., Resistance of seeds to desiccation. Jour. Agric. Research 14:525–532. 1918.

[5] STONE, HERBERT, The ascent of the sap and the drying of timber. Quart. Jour. Forestry 12:261–266. 1918.

[6] REINKING, OTTO A., Philippine economic plant diseases. Philipp. Jour. Sci. 13:165–274. *pls. 20. figs. 43.* 1918.

will be welcomed by plant pathologists, especially those who are interested in tropical plant diseases. In the introduction the author states that the losses due to fungi are at least 10 per cent. He also states that "certain articles on phytopathology in the tropics give an entirely wrong impression of the number and destructiveness of the diseases." In the Malayan regions, at least so far as the Philippines are concerned, there are represented all groups of fungi that are present in the temperate regions. Extremely destructive diseases are produced by some of each group. It is very evident from this and other works that the diseases follow the host plants very closely. Agricultural plants, especially vegetables and truck crops that are very widely distributed, are attacked by the same pathogens, whether grown in the tropical or temperate zones. The coffee industry was at one time wiped out by a fungus, the cacao loss is about 50 per cent, and the rice losses are very heavy. The author lists 60 hosts of which about one-half are grown to a greater or less degree within the bounds of the United States, especially in the southern states or Pacific Coast states. There are a total of 339 diseases listed, many of which are found within the United States. The author gives brief but accurate descriptions of the symptoms, the causal organisms, and statements concerning the control measures. Ten pages are devoted to the discussion of spray mixtures and methods of control.—Mel T. Cook.

Root-nodules.—Miss Spratt[7] has investigated the formation of root-nodules by *Bacillus radicicola*. The plants producing nodules when infected are sharply differentiated into 2 classes, legumes and non-legumes. In the Leguminosae the cortical cells respond to the stimulus, resulting in the nodule. In other plants the penetration of the bacteria into root-hairs and cortex induces no morphological change until a young lateral root is infected during its passage through the cortex, and as a consequence becomes swollen and forms the nodule. In other words, the root-tubercles of non-leguminous plants are modified lateral roots, while those of the legumes are exogenous in origin. A contrast in the structure of the 2 types of nodule is evident. In leguminous nodules the bacteroidal tissue is central, and the vascular system consists of a number of peripheral strands; while in the non-leguminous nodules the stele is central, retaining its connection with the root cylinder and growing point. In making a comparative study of the nodules of Leguminosae, Miss Spratt recognizes 4 types, based chiefly upon the distribution of meristem, bacteroidal tissue, and vascular tissue, and these types are definite enough to characterize various groups of Leguminosae.

The author concludes that "the form of the nodule depends primarily on the nature of the environment of the host, which influences the cell-sap and consequently the behavior of the bacteria after they have entered, and secondarily on the anatomical peculiarities of the particular plant."—J. M. C.

---

[7] Spratt, Ethel R., A comparative account of the root-nodules of the Leguminosae. Ann. Botany **33**:189–199. *pl. 13. figs. 5.* 1919.

Soil fertility.—From a study of the distribution of phosphorus, nitrogen, calcium, potassium, and other elements in soils whose previous history and crop yields are known, certain Rothamsted plots, Pennsylvania State College fertility plots, and Strongsville, Ohio, plots, VAN ALSTINE[8] draws conclusions as to the movement of nutrient elements in the soil.

Phosphorus used as a fertilizer moves but slightly in the soil, remaining fixed until removed by plants, or by the erosive action of water or wind. Certain alkali salts stimulate a larger use of phosphorus, especially by legumes. Potassium also is readily held by the soil, but moves somewhat more freely than phosphorus when other fertilizer salts are used. It may then be carried down beyond the reach of the root system. Nitrogen leaches out into the drainage water to a certain extent, but the loss is small if the crop remains on the soil throughout the growing season. Nitrogen will even accumulate in the soil, mostly in roots and other residues. Carbonates wash out of the soil readily, particularly in the presence of ammonium salts. Magnesium also will leach under this circumstance. The calcium content of the soil decreases as the carbonates go out, and with alkali fertilizers it decreases more rapidly than acidity develops. In the presence of ammonium salts, calcium loss occurs just about as rapidly as acidity develops. Such facts as these should be taken into consideration in developing a rational fertilizer practice.—C. A. SHULL.

Suspensor of Trapa.—TISON[9] has described a remarkable suspensor developed by *Trapa natans*. For some time during embryogeny the suspensor region grows with remarkable vigor, the cells becoming numerous, as well as very large and turgid. The embryo is finally differentiated at the tip of the massive suspensor, which is also the source of nutritive supply to the embryo during its earlier stages. Similar suspensor behavior has been described by GUIGNARD among the Leguminosae, and by TREUB in certain Orchidaceae, but in none of them does the situation seem to be so extreme as in *Trapa*.—J. M. C.

Opuntia.—GRIFFITHS[10] has published 8 new species of *Opuntia*, which he has recognized in connection with cultures of *Opuntia* upon the Department of Agriculture grounds at Chico, California.—J. M. C.

---

[8] VAN ALSTINE, E., The movement of plant food within the soil. Soil Science 6:281–308. 1918.

[9] TISON, M. A., Sur le suspenseur du *Trapa natans* L. Rev. Gén. Botanique 31:219–228. *pl. 4. figs. 5.* 1919.

[10] GRIFFITHS, DAVID, New and old species of *Opuntia*. Bull. Torr. Bot. Club 46:195–206. *pls. 9, 10.* 1919.

BACK VOLUMES

OF

The Botanical Gazette

AT REDUCED PRICES

This is the time to secure back numbers of the *Botanical Gazette.*

Delay may mean failure to complete your set as the numbers are becoming more difficult to find, while a complete set is rare.

OUR OFFER

Until December 31, 1919, we will sell a limited number of back volumes and single copies at a reduction of 25 per cent from the published prices.

SEND US YOUR ORDER NOW

We have also files of the *Astrophysical Journal* and the *Journal of Geology.*

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - ILLINOIS

It Gives the Typist More Time to Type

These three things—***inserting the paper, typing, line spacing,*** are all the typist has to do when she operates the

SELF-STARTING REMINGTON

Hand settings of the carriage, which are necessary on all other typewriters, are eliminated absolutely—and that means a time saving of 15 to 25 per cent in transcribing business letters. The Remington Self-Starter, or automatic indenter, gives the typist more time to type. That means a bigger day's work with no more effort; bigger typewriter value at no greater price.

REMINGTON TYPEWRITER COMPANY
(Incorporated)
374 Broadway New York

FINE INKS AND ADHESIVES
For those who KNOW

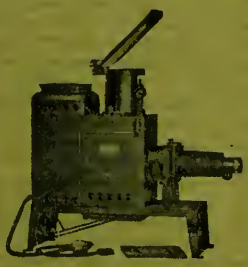
Higgins'
- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

At Dealers Generally

CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street Brooklyn, N.Y.



Combined Balopticon for Lantern Slides and Opaque Objects. Price, $150.

Bausch and Lomb
BALOPTICON
THE PERFECT STEREOPTICON

Education through the Eye

Which "gets" a thought "across" faster—word or picture?

Why are "movies" quicker educators than books?

Why are "illustrated" talks better attended than **all-words** lectures?

Visual education—education through Bausch & Lomb Balopticons, particularly—is recognized now as the quickest means for making people understand.

Didn't **pictures** bring the war to us faster, more vividly, than words?

And as for the quality of Balopticon pictures—Balopticons are Bausch & Lomb products. That guarantees the quality of the pictures.

Bausch & Lomb Optical Co.
596 ST. PAUL STREET ROCHESTER, N.Y.
NEW YORK WASHINGTON CHICAGO SAN FRANCISCO

Leading American Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

Volume LXVIII Number 5

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

NOVEMBER 1919

Chemical Constituents of Amaranthus retroflexus. Contributions from the Hull Botanical Laboratory 254 - - - - - M. L. Woo 313
(With eleven figures)

Staminate Strobilus of Taxus canadensis. Contributions from the Hull Botanical Laboratory 255 - - - - - A. W. Dupler 345
(With Plates XXIV–XXVI and twenty-two figures)

Colloidal Properties of Bog Water - George B. Rigg and T. G. Thompson 367

Vegetation of Undrained Depressions on the Sacramento Plains Francis Ramaley 380
(With one figure)

Briefer Articles

Aaron Aaronsohn - - - - - - - - - J. M. C. 388
(With portrait)

Current Literature

Book Reviews - - - - - - - - - - - 390

Notes for Students - - - - - - - - - - 391

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London and Edinburgh*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Volume LXVIII Number 5

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued November 17, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, £1 12*s.* 6*d.* each; single copies, including postage, 3*s.* 6*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 13 North Szechuen Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

VOLUME LXVIII NUMBER 5

THE
BOTANICAL GAZETTE

NOVEMBER 1919

CHEMICAL CONSTITUENTS OF AMARANTHUS RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 254

M. L. Woo

(WITH ELEVEN FIGURES)

Introduction

It is a well known fact that weeds retard the development of cultural plants. This is due to a number of causes: use of water, shading, use of nutrient salts, etc. It has been claimed for various species of *Amaranthus* that they not only absorb nitrates to care for their nutrient needs, but that they store much nitrogen as nitrate. If this be true, this genus has an excellent adaptation to enable it to combat cultural plants, for nitrate supply is a common limiting factor for crop growth. In order to investigate this statement, to locate the place of nitrate storage, and to determine the amount of nitrogen used otherwise by this plant, separate analyses were made of roots, stems, leaves, and branches of *Amaranthus retroflexus* at various stages and under various conditions of growth. The amount of the several carbohydrates was also determined in each analysis, in order to calculate the carbohydrate-nitrogen ratio which is lately receiving so much attention. A tissue analysis of the seeds was also made in an endeavor to ascertain more fully the chemical constituency of this plant, with the hope of learning more of the peculiar germinative behavior of these seeds.

Historical

The first chemical analysis of *Amaranthus* was made by BOUTIN (1), a French chemist, in 1873. Housewives of that time were using these plants to clean their cooking utensils. This fact gave it some commercial importance and brought it to the attention of chemists. It was thought that the ability to "cut grease" must be due to acids in the plant. To verify this BOUTIN incinerated 100 gm. dry weight of the entire plant, and obtained 16 gm. residue. Water was added to leach out the soluble salts. The soluble portion weighed 8 gm. He called this potassium carbonate, and calculated the equivalent weight in grams of potassium nitrate, and found it to be 11.68, or 11.68 per cent. BOUTIN concluded that this plant was neutral in reaction on account of the presence of the neutral salt KNO_3 (as a matter of fact this plant is acid in reaction). Later (2) he made analyses of the other species of *Amaranthus* by the same method, to determine the amount of KNO_3. The result of his analysis was as follows: *A. atropurpureus* contains 22.77 per cent KNO_3 (one kilogram gives 31 gm. N and 103.5 gm. K); *A. Blitum* contains 11.68 per cent KNO_3; *A. ruber* contains 16 per cent KNO_3 (one kilogram gives 22 gm. N and 72 gm. K). It is evident that BOUTIN'S method is not an accurate quantitative method of determining the amount of nitrate present. On the other hand, he demonstrated by other qualitative methods that the several species of *Amaranthus* studied contain a large amount of potassium nitrate. BROSSET (3) suggests the use of these plants as a fertilizer.

PAMMEL and DOX (15), in 1917, made microchemical tests of three common pigweeds, *A. blitoides*, *A. graecizans*, and *A. retroflexus*, and found them to contain abundant starch, some protein, and a little fat. In addition they made the Kjeldahl-Gunning nitrogen determination and found these species to have 1.88, 2.32, and 2.49 per cent of nitrogen. Multiplying by the factor 6.25, they obtained 11.75, 14.52, and 15.59 per cent of protein respectively.

HARDING and EGGE (8) made an analysis of the seeds of *A. retroflexus* for fats, protein, starch, sugars, hemicellulose, crude fiber, and tannin.

Of late much significance is being attached to the carbohydrate-nitrogen ratio of plant tissues, or, as FISCHER (6) puts it, the $\frac{C}{N}$. On the basis of work done by him and others, FISCHER makes the following generalizations. If the value of $\frac{C}{N}$ rises by an increase in the amount of carbon, or by a decrease in the amount of nitrogen furnished the plant, there is an increase in the amount of flowering. If the value of $\frac{C}{N}$ drops by a decrease in the amount of carbon, or by an increase in the amount of nitrogen furnished the plant, there is an increase of vegetative growth and a reduction of flowering. Briefly stated, a great preponderance of carbohydrates in plants favors flowering. Since the carbon of plants is fixed from the carbon dioxide of the air by photosynthesis, conditions that favor photosynthesis will tend to increase the ratio, and according to FISCHER the flower production. He found that increased partial pressures of carbon dioxide in the air had this effect. Since nitrogen is absorbed from the soil in the form of nitrates, conditions that favor nitrate absorption will decrease the ratio, and according to FISCHER favor vegetation.

KRAUS and KRAYBILL (12), on the basis of much more critical work, including numerous cultures, tissue analyses, and microchemical and anatomical studies, conclude that a very high $\frac{\text{carbohydrate}}{N}$ value gives little vegetation and little or no reproduction; a medium $\frac{\text{carbohydrate}}{N}$ value gives moderate vegetation and good reproduction; and a low $\frac{\text{carbohydrate}}{N}$ value gives vigorous vegetation and little reproduction. Through their extreme conditions of culture, withholding nitrates, it is probable that KRAUS and KRAYBILL got much higher carbohydrate plants than FISCHER obtained in his cultures, hence their conclusion that very high $\frac{\text{carbohydrate}}{N}$ gives little vegetation or reproduction. In short, FISCHER worked only on the portion of the $\frac{C}{N}$ curve that induced fair vegetation and good reproduction or extreme vegetation and little reproduction, but not on the extreme of the curve that greatly

reduces both vegetation and reproduction. KRAUS and KRAYBILL cite literature showing that various conditions that greatly retard growth produce high carbohydrate plants. It seems that such conditions retard the use of carbohydrates for building new tissue to a greater degree than they do photosynthesis, and thereby lead to an accumulation of carbohydrates.

HEDLUND (9) finds that under like cultural conditions those varieties of winter wheat that have a higher percentage dry weight in the autumn are generally more winter hardy than the ones having a low percentage dry weight, and that cultural conditions that make for high percentage dry weight in any variety also make for winter hardiness. He finds, as do KRAUS and KRAYBILL, that high percentage dry weight is due to high percentage carbohydrate, and therefore high $\frac{\text{carbohydrate}}{\text{N}}$.

RIBERA (16) finds that all cultural conditions that increase the percentage dry weight in wheat decrease lodging. From this and the two investigations previously mentioned it is evident that high $\frac{\text{carbohydrate}}{\text{N}}$ increases straw strength and decreases lodging. High percentage of carbohydrate is said to increase hardiness, at least in part, by the greater amount of glucose present, and it may increase straw strength by inducing greater development of mechanical tissue along with greater thickness of walls, as KRAUS and KRAYBILL found for certain tissues of the tomato.

Methods and results

GREEN PLANT AT VARIOUS STAGES OF DEVELOPMENT

Preparation of samples.—Samples were secured on June 3, June 20, and July 8 consecutively from a vacant lot on 59th Street and Ingleside Avenue, Chicago. On June 20 samples were taken from two places, namely, the manure pile (rich soil) and the knoll (poor soil) for comparative work. The soil particles adhering to the roots and rootlets were removed by running water from a filter pump. As the velocity of water was very great, the soil particles were removed without difficulty. The roots were partially dried by the air current from the laboratory air line, and

finally dried by the use of paper towels. The roots, stems, and leaves were detached for separate analysis. The roots and the main stems were separated by cutting just between the cotyledon scar and the first branching rootlet. The leaf blades with the petioles were separated from the stem. Each portion was weighed and the length and diameter measured. Table I gives a brief description of the three consecutive samples.

TABLE I

THE GREEN PLANT AT VARIOUS STAGES OF GROWTH

Measurement	June 3 collection	June 20 collection	July 8 collection
	Inches	Inches	Inches
Average height	1.00–4.00	6.00–8.00	20.00
Taproot length	1.00–1.50	4.00–6.00	4.00– 6.00
Taproot diameter	.....	0.10–1.00	1.00– 1.20
Secondary rootlets length	None	0.10–1.00	8.00–14.00
Secondary rootlets diameter	None	.....	0.13– 0.25
Stems length	1.00–4.00	6.00–8.00	20.00
Stems diameter	0.13–0.25	0.37–0.75	0.13– 0.25
Lateral branches length	None	0.10–1.50	14.00
Lateral branches diameter	None	.....	0.25– 0.50
Seed head	None	None	0.25– 1.00
Green weight	Grams	Grams	Grams
Roots	26.00	28.50	29.20
Main stems	52.05	97.40	88.30
Branches	None	None	80.45
Leaves	188.90	142.20	110.25

The green samples were then immediately put in a freezing chamber, allowed to freeze overnight, and ground in a meat grinder the next morning. The freezing prevents losses. In the frozen condition no juices ooze out or spatter in the manipulation. The samples were boiled with 95 per cent alcohol to destroy the enzymes, and were then transferred to extraction cups, with filter paper thimbles, previously dried and weighed. The tissues were fractionated according to KOCH'S (11) scheme for tissue analysis, namely, the lipin or ether soluble fraction (F_1), the alcohol water soluble fraction (F_2), and the insoluble fraction (F_3). In the green plant F_2 was comparatively small, consisting of chlorophyll and extracts of various pigments. The F_1 was put together with F_2 for the following carbohydrate and nitrogen estimation.

ANALYSES

Nitrogen compounds

NITRATES.—The nitrates were determined by the Schlösing-Wagner method (14) as modified by KOCH for use in his laboratory. The modification consists essentially in the use of an inverted burette instead of a tube sealed at one end, and of the Van Slyke apparatus (only volumetric tube and Hemple pipette), to measure the true volume of nitric oxide. The principle of the method is: $3Fe^{++}+NO_3^-+4H^+ \rightarrow 3Fe^{+++}+NO\,gas+2H_2O$. Therefore 1 mol. NO gas gives an equivalent of 62 gm. of NO_3, or 1 cc. of gas $= \frac{62}{22400} = 2.77$ mg. NO_3.

In order to determine the accuracy of this method, a known solution of KNO_3 (0.5 per cent) was used. Four consecutive determinations with 10 cc. of the known solution were made. The average volume of nitric oxide gas for each 10 cc. solution, calculated to standard condition, was 11.12 cc. The theoretical volume for 10 cc. of 0.5 per cent KNO_3 is 11.078 cc.

The determination of nitrates in the samples was made by taking an aliquot of the soluble fractions (F_1 and F_2). The nitric oxide gas driven over was caught in an inverted burette which had previously been filled with 40 per cent NaOH to absorb the CO_2 and neutralize the hydrochloric acid (HCl gas will come over when the hydrochloric acid concentration reaches 20 per cent in the boiling flask). The burette containing the nitric oxide gas was set aside and allowed to cool to room temperature; then the nitric oxide gas was transferred to the Van Slyke apparatus. The total volume and the volume of unabsorbed gas were recorded. The absorbed volume by the alkaline $KMnO_4$ in Hemple pipette is that of nitric oxide. This volume was then calculated to standard volume from temperature and barometric pressure, for example:

	I	II
Aliquot in cc. used	25.0	25.0
Total volume of gas (nitric oxide+air)	4.80	4.85
Volume of unabsorbed gas (air)	1.27	1.26
Volume of (absorbed) nitric oxide	3.53	3.59
Barometric pressure = 746.9; temperature 20.5° C.		
Volume at standard condition	3.23	3.27

	I	II
Equivalent in milligrams of NO_3	8.95	9.06
Milligrams of dry substance (25 cc.) used	107.75	107.75
Percentage NO_3 in soluble fractions F_1+F_2	8.30	8.41
The percentage soluble fractions in whole samples	45.9	45.9
Therefore percentage of NO_3 calculated on whole sample	3.81	3.86

Soil samples were taken at the same time that the green plants were gathered. The nitrates were estimated by the colorimetric method with phenoldisulphonic acid. The moisture in percentage and nitrates in parts per million are shown in fig. 1.

On June 20 the samples from the manure pile and from the knoll were taken for comparison. The nitrate content of the soil and that in the plant were as follows:

	Knoll sample	Manure pile sample
NO_3 in ppm in soil	300	29
NO_3 in percentage in plant (stem only)	1.71	1.45

The high NO_3 content in the soil of the knoll sample is probably due to the fact that some one, perhaps the gardener, had disturbed the soil by dragging his cultivator over it accidentally in cultivating his plot near by. The second reason is the better drainage and aëration in the knoll, and therefore better conditions for nitrification; but the striking fact is that high nitrate content in the soil did not bring about a proportional high nitrate content in the green plant organs. The rate of absorption increases with the aging of the plant; when the plants were about 25 days old, the nitrate in the stem was only 1.71 per cent. Eighteen days later (July 8) the nitrate content had risen to 8.58 per cent. During the same period branches grew from 0.1 to 14 inches, and their nitrate content rose to 12.50 per cent. This rapid increase in absorption of nitrates may partially be explained by the increase in extent of the absorbing roots from a radius of a few inches to about 2 ft.

The nitrate content in the roots, stems, and leaves is given in table II and is also shown in fig. 1. The nitrate content of the roots falls gradually from 1.85 per cent on June 3 to zero on June 20. At the same time nitrates in the leaves fall from 1.38 per cent to zero, while in the stem there is a gradual increase. There must be a definite reason for such differences. The differences may be due

to many causes. In the leaves protein synthesis is going on continuously in the presence of soluble carbohydrates. There is also synthesis of other organic nitrogen compounds, such as chlorophyll,

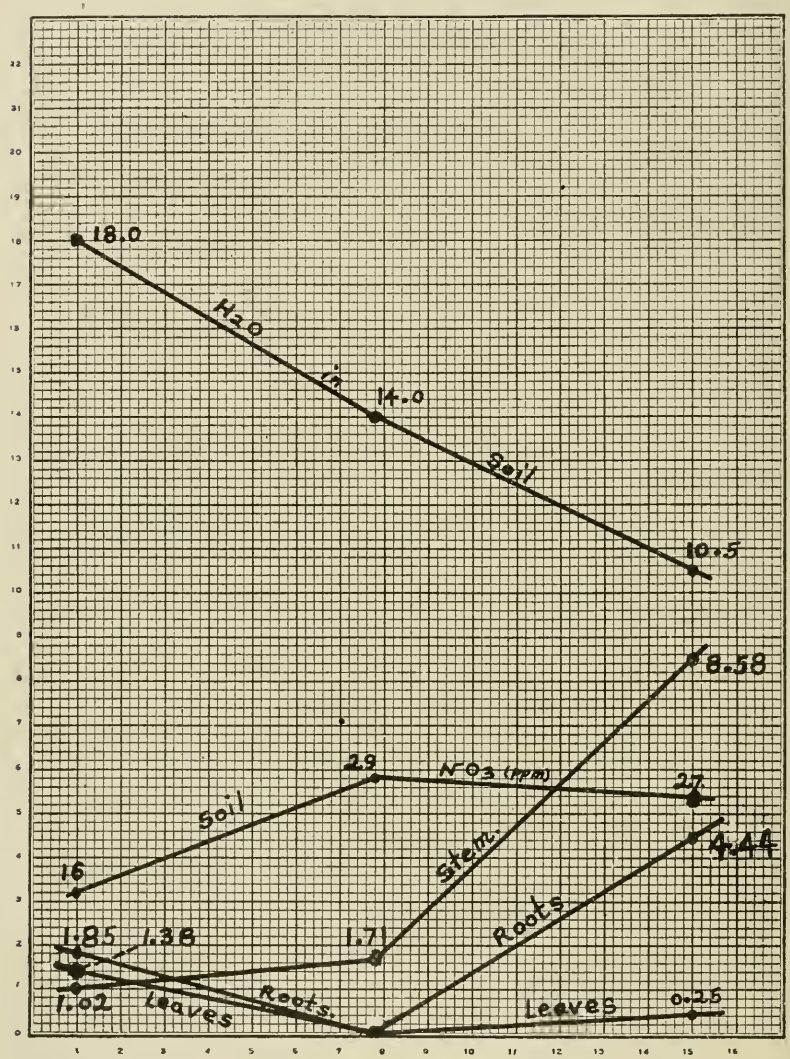


FIG. 1.—Relation of soil nitrification and nitrate intake by green plant organs; nitrate in soil expressed in parts per million; all other data calculated as percentage on dry weight basis.

phospholipins, etc., and all of the nitrates of the leaves seem to be used up in these syntheses. The nitrates are carried from the roots to other parts of the plants as fast as they are taken up from the soil. There may be as high a concentration of nitrates in the roots as in the soil (29 parts per million). This low concentration cannot be estimated by this method and would therefore be missed.

The stem and branches are the primary nitrate storage organs. The nitrate content rises as high as 8.58 per cent in the stem and 12.5 per cent in the branches during the early seed formation period. This high content is shown still more clearly by the ratio of nitrate nitrogen to the total nitrogen. This is 32.8 per cent for the roots, 51.85 per cent for the stems, 56.4 per cent for the branches, but only 1.25 per cent in the leaves. Curves showing this ratio in these organs at different stages are given in fig. 2. This large supply of nitrates in the stem and branches may be drawn upon heavily for further growth and seed production, although the supply seems more than adequate for these uses. There is also no reason for thinking that nitrate absorption ceases at this time. The extent to which this storage of nitrate is drawn upon by later development could be ascertained by the analysis of a set of samples taken late in the fall when seed formation and growth were complete. It is to be regretted that circumstances made such an analysis impossible for this paper.

It is worthy of note that the nitrate storage organs are the ones that made the most rapid growth in length, weight, and volume. The stem which rose from 8 inches on June 20 to 20 inches on July 8 at the same time increased in nitrate content from 1.71 to 8.58 per cent on dry weight basis. In addition to the stem there are numerous side branches which elongated from 0.1 to 14 inches in 18 days, making nearly 1 inch in 24 hours. At the same time there was an increase in percentage of nitrates per gram of dry matter from 1.71 on June 20 to 12.50 per cent on July 8. The rate of nitrate intake per gram per hour seems to follow a geometrical progression in each individual plant.

It appears that *Amaranthus* may be a very considerable factor in depleting soils of their nitrates. Also in case the weeds are burned the nitrogen stored is permanently lost from the soil. It

is a point of interest to know how generally this great power of absorbing and storing nitrates is possessed by weeds.

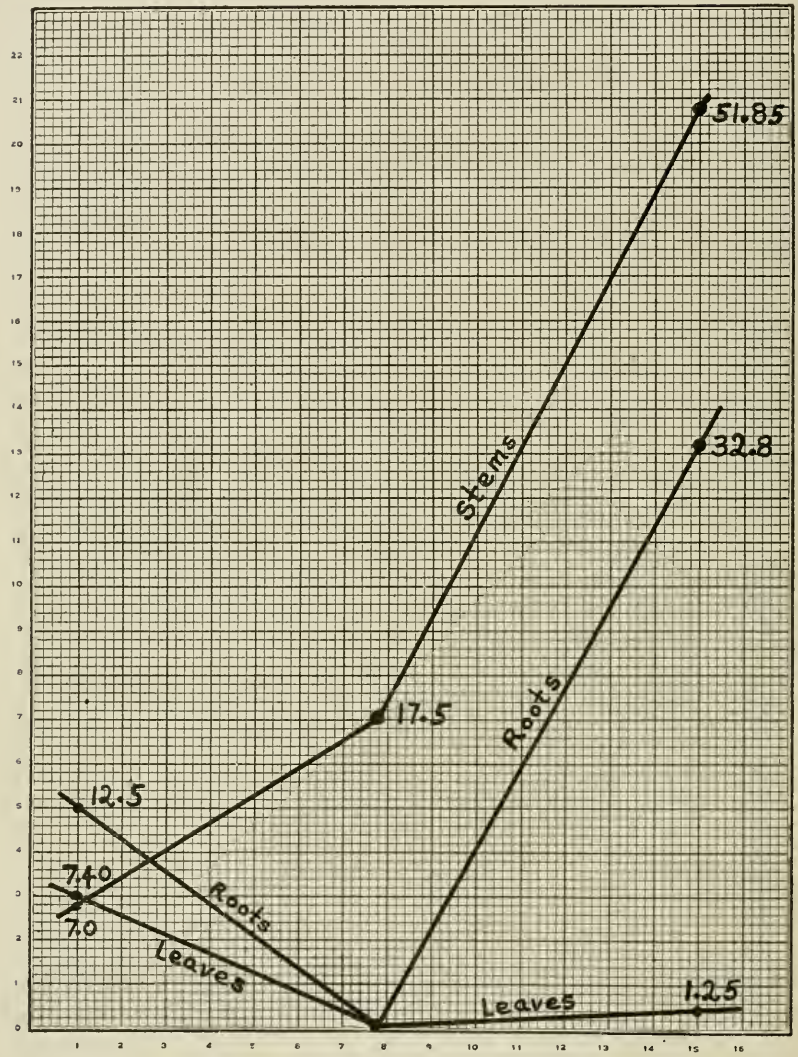


FIG. 2.—Ratio of nitrate nitrogen to total nitrogen; ratio for branches (not shown in figure) 56.4.

AMINO N.—The amino nitrogen was determined by the Van Slyke apparatus. The amino acids thus determined are chiefly

of the mono-amino-monocarboxylic acids. In each estimation only 2 cc. of the solution was used. The amino acid nitrogen deter-

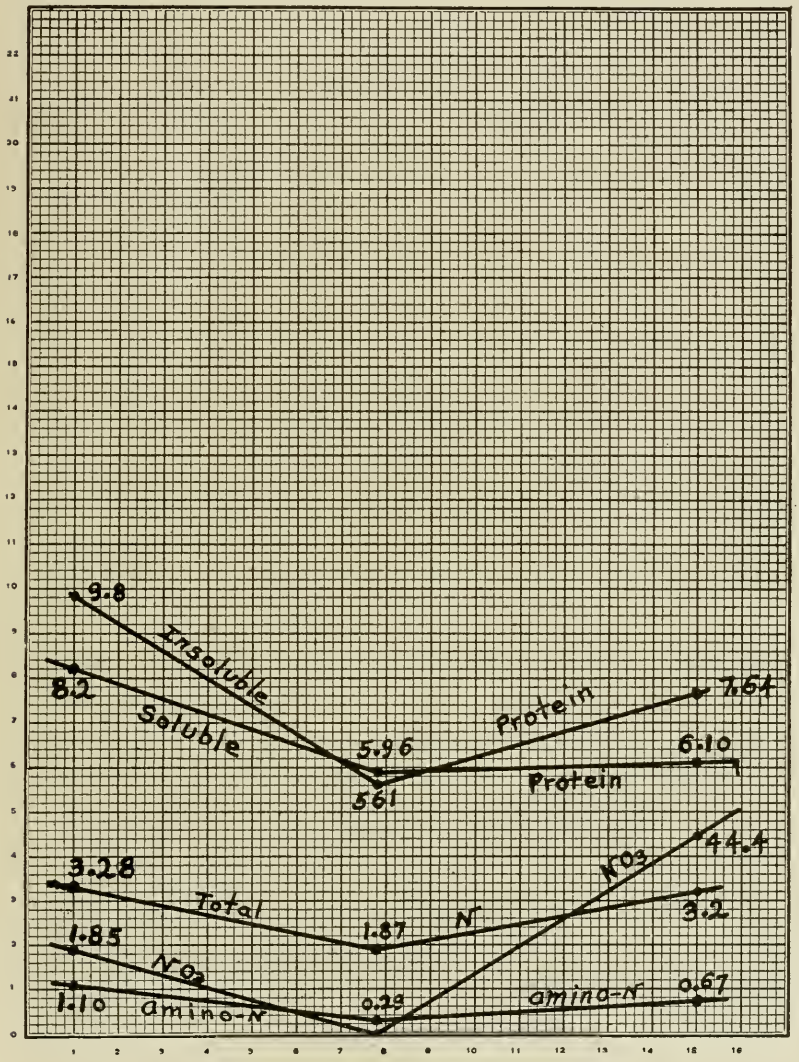


FIG. 3.—Nitrogen compounds in roots

mined throughout the season is given in table II. Curves showing the variation in roots, stems, and leaves are given in figs. 3, 4, and 5 respectively. In all the three organs the variation is very small.

In general the amino N varies directly with the insoluble protein; with a high protein content, high amino N; and low protein content, low amino N.

TABLE II

THE NITROGEN COMPOUNDS IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total N	3.33	3.33	3.19	3.21	....	....	4.25	4.27
Nitrates NO_3	1.77	1.93	0.99	1.04	....	....	1.36	1.40
Nitrate N	0.40	0.43	0.22	0.23	....	....	0.31	0.32
Amino N	1.09	1.13	0.43	0.43	....	....	0.97	0.96
Insoluble N	1.62	1.52	1.74	1.73	....	....	3.21	3.23
Insoluble protein	10.18	10.25	10.92	10.80	....	....	20.20	20.30
Soluble protein	8.24	8.17	7.67	7.92	....	....	4.58	4.52
	June 20 collection (6–8 inches)							
Total N	1.85	1.88	2.39	2.21	....	....	4.56	4.51
Nitrates NO_3	None	None	1.67	1.75	....	....	None	None
Nitrate N	None	None	0.38	0.40	....	....	None	None
Amino N	0.29	0.29	0.19	0.19	....	....	1.38	1.37
Insoluble N	0.86	0.91	0.95	0.97	....	....	3.66	3.59
Insoluble protein	5.40	5.72	5.86	6.10	....	....	23.00	22.6
Soluble protein	5.92	6.10	5.28	5.41	....	....	5.66	5.78
	July 8 collection (20 inches)							
Total N	3.21	3.12	3.74	3.80	5.04	4.94	4.85	4.80
Nitrates NO_3	4.41	4.47	8.40	8.75	12.50	12.40	0.25	0.246
Nitrate N	0.99	1.02	1.90	1.98	2.85	2.80	0.06	0.06
Amino N	0.63	0.70	0.35	0.34	0.48	0.49	1.44	1.42
Insoluble N	1.29	1.14	0.95	0.91	1.01	0.98	3.29	3.21
Insoluble protein	8.10	7.17	5.96	5.71	6.35	6.15	20.62	20.08
Soluble protein	6.16	6.05	5.60	5.70	7.42	7.30	9.42	9.62

INSOLUBLE PROTEIN.—The insoluble protein was calculated from nitrogen of the insoluble fraction (F_3). The insoluble protein is given in table II, and curves showing fluctuation during the growing season are given in figs. 3–5. The insoluble protein falls and then rises again at maturity in the root, while in the leaves the fluctuation is in the opposite direction (see curves). In the stem the decline is in the early stage from 10.89 per cent (June 3) to 6.03 per cent (June 20). From that time on the curve is almost a straight horizontal line; therefore the rate of synthesis of the insoluble protein must have been keeping pace with the growth of the stem,

because the fall at this time is only from 6.03 to 5.84 per cent (almost within experimental error).

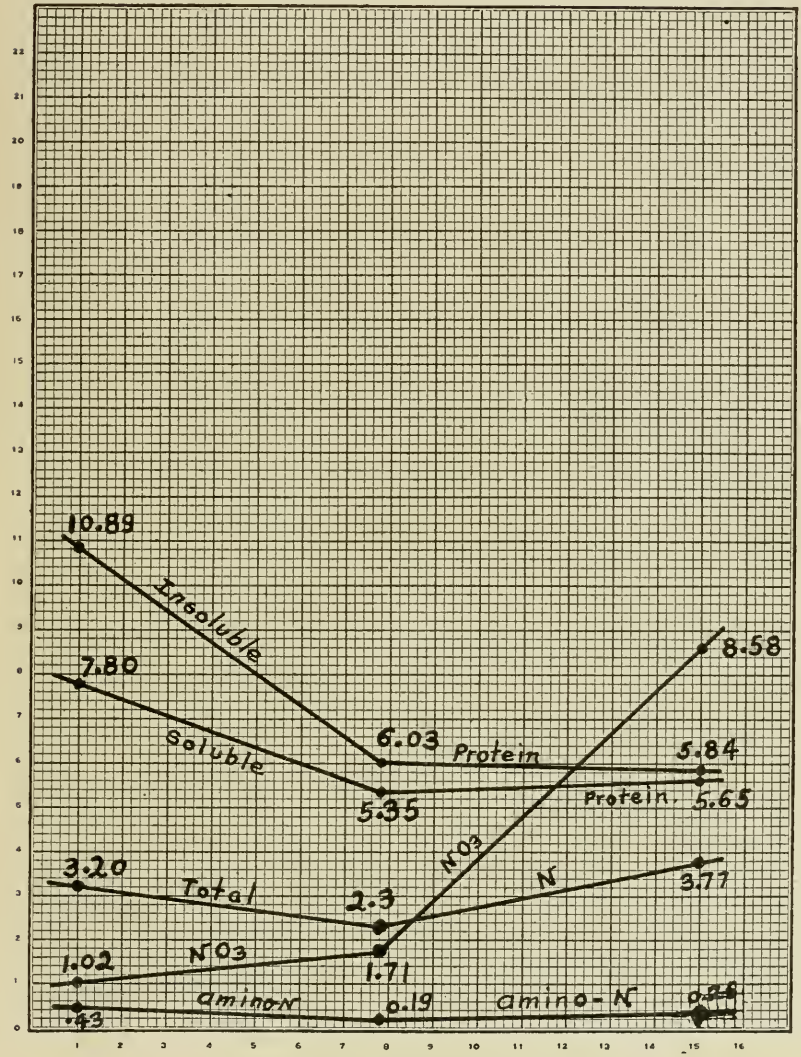


FIG. 4.—Nitrogen compounds in stems

SOLUBLE PROTEIN.—Soluble protein in F_1 and F_2 is computed from the Kjeldahl nitrogen determination. The nitrate nitrogen

was determined separately (by the method previously described); so the Kjeldahl determination was conducted without any modifica-

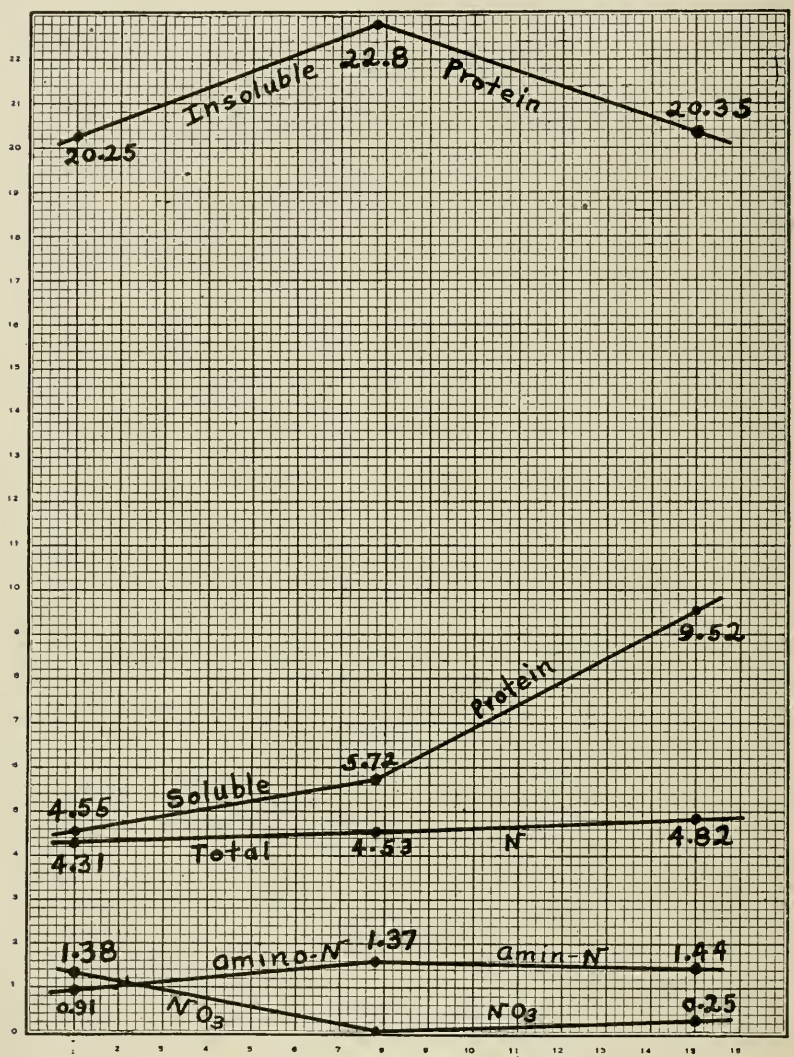


FIG. 5.—Nitrogen compounds in leaves

tion for nitrates. (Previously zinc was used, but this did not reduce any nitrate. Experiment with a known solution 0.5 per cent KNO_3,

per official method by the use of salicylic acid and sodium thiosulphate, reduced only 60 per cent of the nitrate into ammonia.)

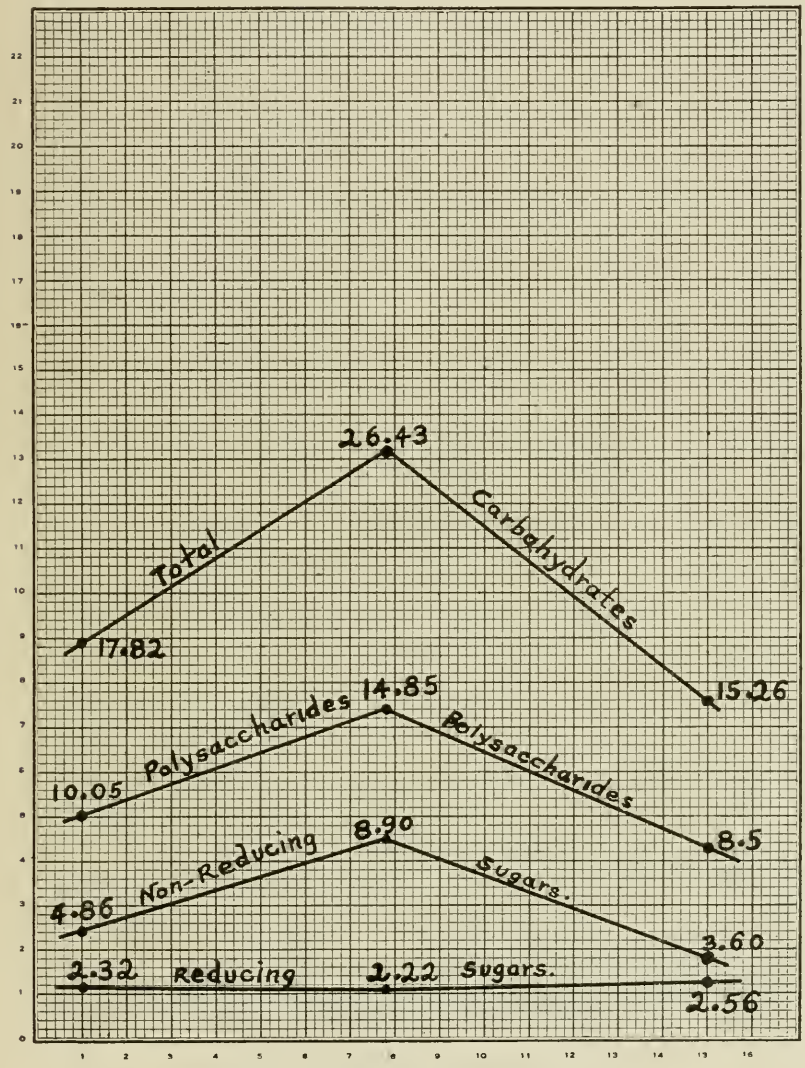


FIG. 6.—Different carbohydrates in roots

It is incorrect to call all this nitrogen as derived from protein, because part of the soluble nitrogen was from the breaking down

of the chlorophyll; but for comparison it is not out of place to calculate the N by the factor 6.29 to convert it into soluble protein

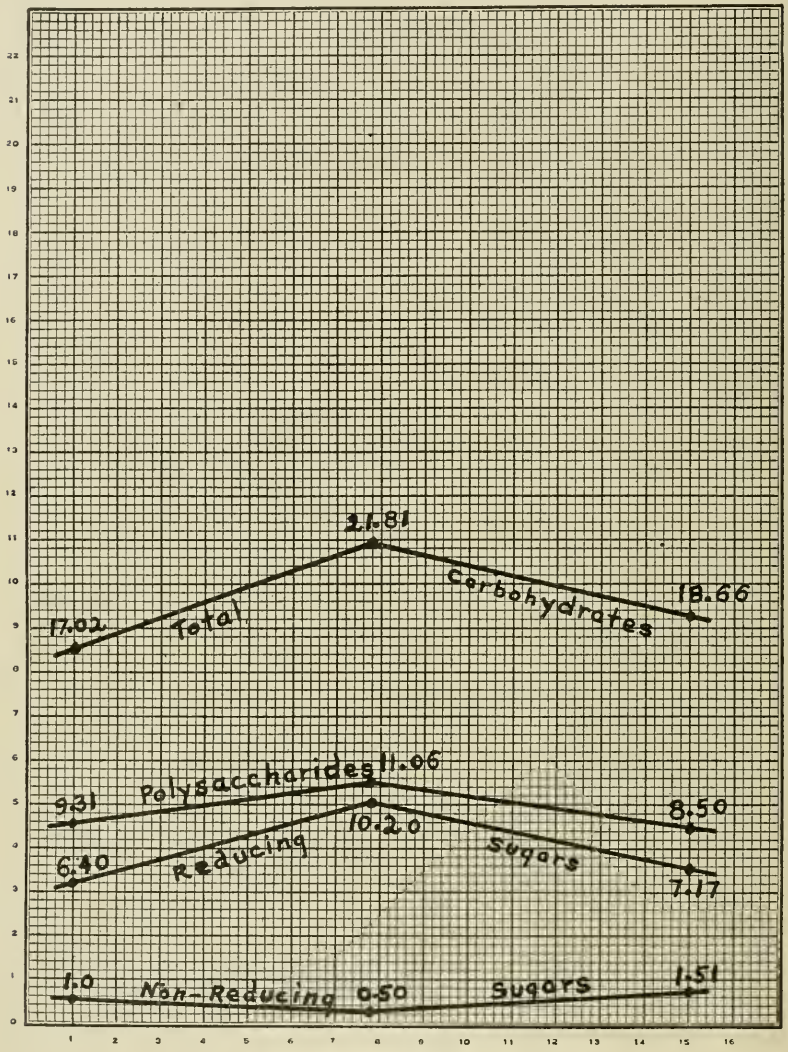


FIG. 7.—Different carbohydrates in stems

for temporary convenience in interpreting the results. The curves of the soluble protein in figs. 3–5 are self-explanatory, showing the variation throughout the season.

Carbohydrates.—The carbohydrates were determined by the reduction method with Fehling solutions. The cuprous oxide

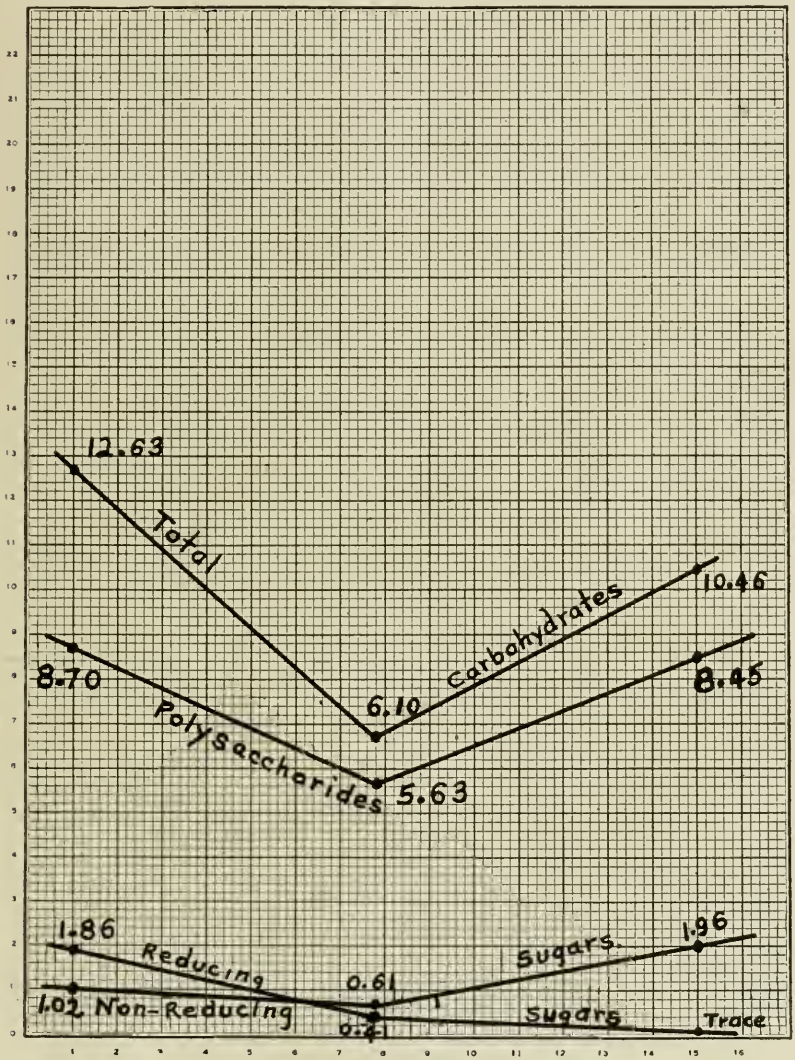


Fig. 8.—Different carbohydrates in leaves

obtained was dissolved in excess of ferric ammonium sulphate with H_2SO_4 previously added. The ferrous ions produced by the oxidation of cuprous oxide were titrated against a N/20 $KMnO_4$

solution, in which 1 cc. represents 3.1 mg. of copper. The corresponding equivalents of the different sugars expressed in milligrams were found in the Munson-Walker table. The weight of sugar found divided by the material used gives the amount of sugar contained in 1 gm. of material.

The soluble carbohydrates are in F_1 and F_2. The reducing sugar was first determined. The non-reducing sugar was obtained by subtracting the reducing sugar from the total sugar by hydrochloric acid hydrolysis at 67–69° C. for 10 minutes.

The insoluble carbohydrates are in F_3. They consist essentially of colloidal polysaccharides, the greater part of which was starch. The polysaccharides were determined by the Fehling solution after acid hydrolysis for 2.5 hours with a reflex condenser.

TABLE III

THE CARBOHYDRATES IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total carbohydrates	17.81	17.83	16.95	17.09	....	.....	12.76	12.57
Reducing carbohydrates	2.38	2.27	6.51	6.30	....	.....	1.86	1.87
Non-reducing	5.43	5.46	1.16	1.44	....	.....	2.14	2.05
Polysaccharides	10.00	10.10	9.28	9.35	....	.....	8.76	8.65
	June 20 collection (6–8 inches)							
Total carbohydrates	26.29	26.57	21.78	21.84	....	.....	6.78	6.61
Reducing carbohydrates	2.24	2.19	10.25	10.15	....	.....	0.44	0.38
Non-reducing	9.30	9.43	0.53	0.57	....	.....	0.63	0.67
Polysaccharides	14.75	14.95	11.00	11.12	....	.....	5.71	5.56
	July 8 collection (20 inches)							
Total carbohydrates	15.32	15.21	18.69	18.63	15.18	15.07	10.51	10.41
Reducing carbohydrates	2.85	2.54	7.15	7.20	5.00	5.10	Trace	Trace
Non-reducing	3.82	3.71	1.67	1.50	1.18	1.02	1.96	2.01
Polysaccharides	8.92	8.96	9.87	9.93	9.00	8.95	8.55	8.41

The percentage of the different carbohydrates of various organs estimated at different times throughout the growth period is tabulated in table III. Curves showing the changes in different sugar content in these organs are given in figs. 6–8. These curves show that in the roots the reducing sugars remain constant, while the

non-reducing sugars fluctuate throughout the season. This is just the reverse of what is found in the stems. In the leaves the

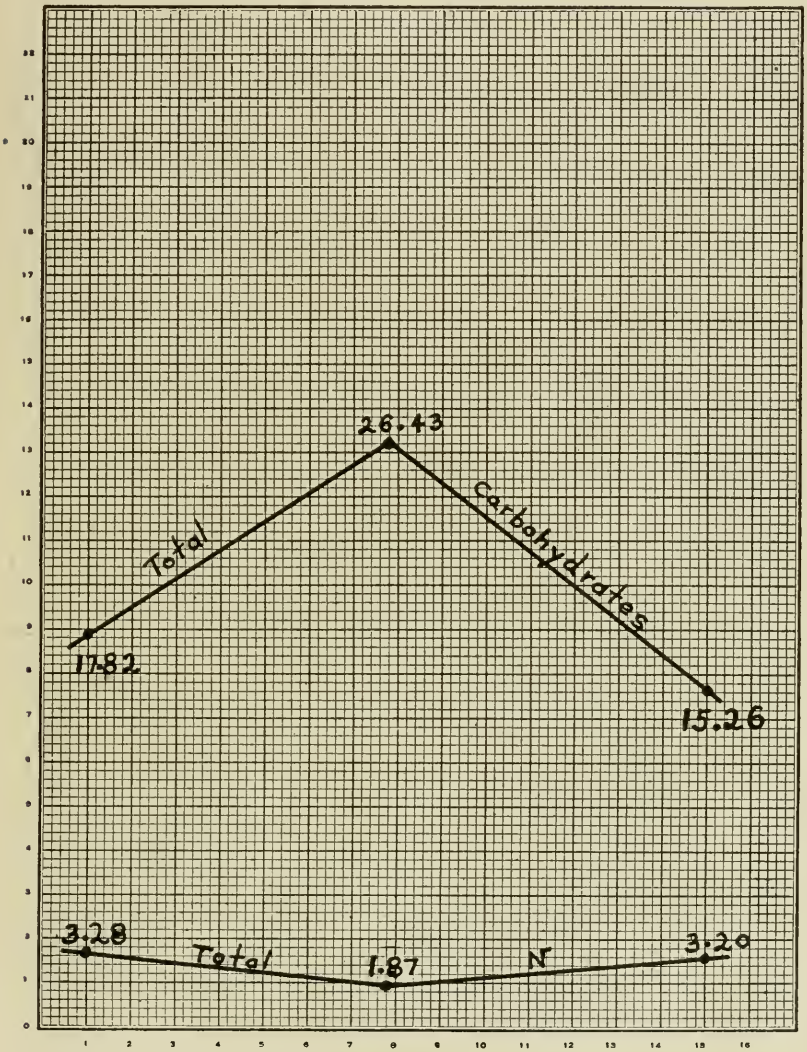


FIG. 9.—Reciprocal fluctuation of carbohydrate and nitrogen in roots (cf. figs. 3 and 6).

fluctuation of the reducing and non-reducing sugars is in the opposite direction; when the reducing sugars are high, the non-reducing

sugars are low, and the reducing type falls to zero at the time of seed formation.

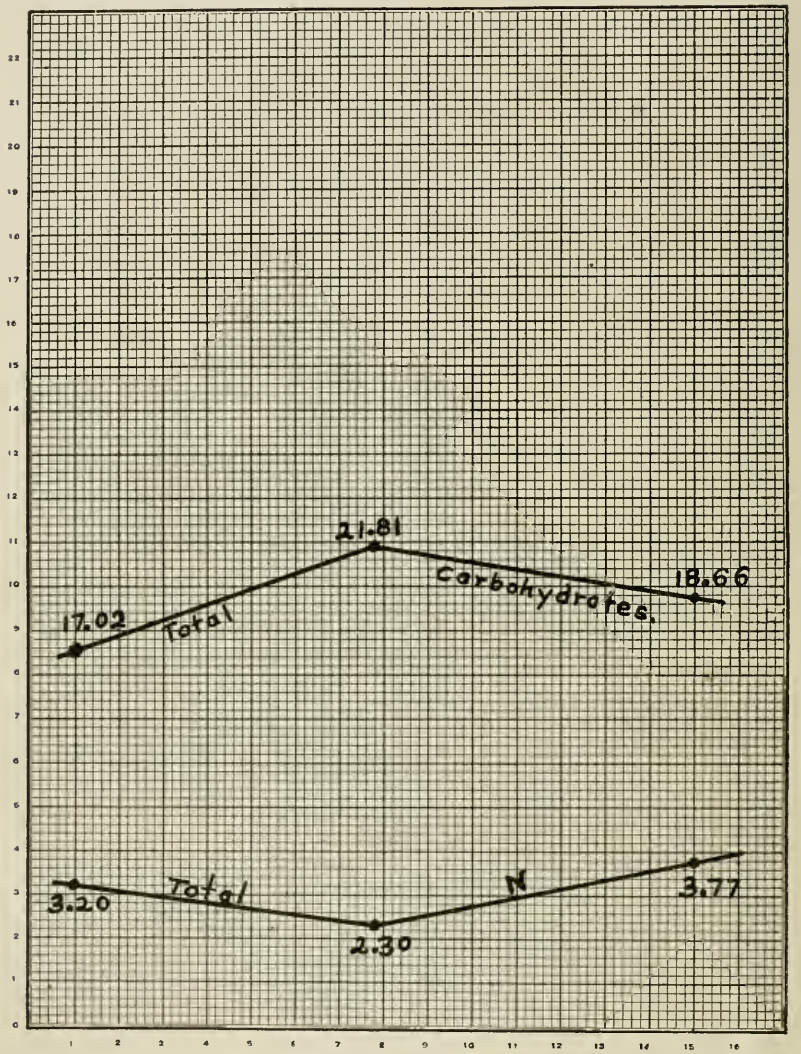


FIG. 10.—Reciprocal fluctuation of carbohydrate and nitrogen in stems (cf. figs. 4 and 7).

CARBOHYDRATE-NITROGEN RATIO.—According to the work of KRAUS and KRAYBILL on the tomato, high nitrogen in a plant is

accompanied by low carbohydrate. "Whatever the conditions under which a plant has been grown, considering the whole plant

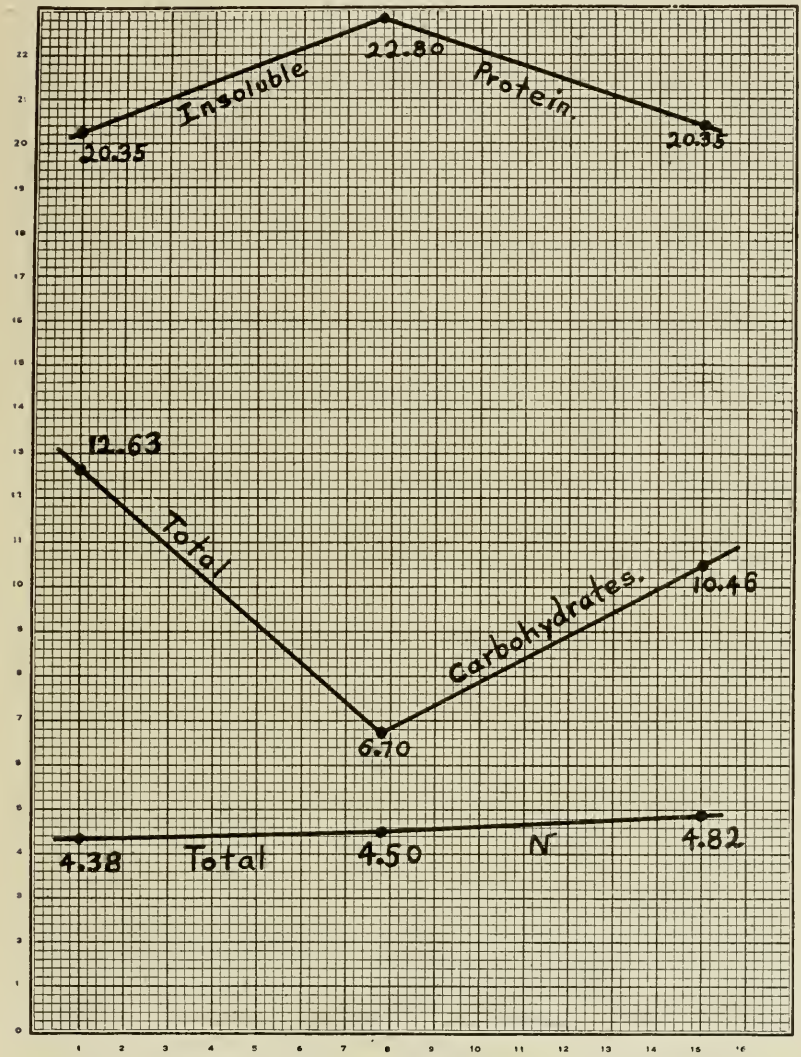


FIG. 11.—Reciprocal fluctuation of carbohydrate and nitrogen in leaves (cf. figs. 5 and 8); notice that carbohydrate reciprocates with insoluble protein and not with total nitrogen.

as a unit, increased total nitrogen and more particularly increased nitrate nitrogen are associated with increased moisture and

decreased free-reducing substances, sucrose, polysaccharides, and total dry matter."

In the work with *Amaranthus* plants I have found a similar situation so far as the relation between nitrogen and carbohydrate is concerned; that is, low nitrogen is accompanied by high carbohydrate and high nitrogen by low carbohydrate. Upon computing the reciprocal condition in the different fractions I find that the product of carbohydrate by nitrogen is not a mathematical constant, but that it varies considerably, sometimes decreasing as the development of the plant progresses. The product varies least in the stem and roots.

Let C_1, C_2, and C_3 be the carbohydrates and N_1, N_2, and N_3 denote the nitrogen, the sub-numbers representing the time of collection. If the carbohydrate and nitrogen hold a reciprocal relation, then $\frac{C_1}{C_2}=\frac{N_2}{N_1}$, $\frac{C_2}{C_3}=\frac{N}{C_2}$, and $\frac{C_3}{C_1}=\frac{N_1}{C_3}$; by clearing the fractions, $C_1 \times N_1 = C_2 \times N_2 = C_3 \times N_3$, etc., or carbohydrate × nitrogen = constant K. Applying this principle, the following constants are obtained.

Insoluble fraction (F_3)

	June 3	June 20	July 8	Av. K.
Roots	15.7	13.06	10.92	13.23
Stems	16.20	10.62	9.20	12.01
Leaves	28.00	20.4	27.6	25.30

Soluble fractions (F_1+F_2)

Roots	10.10	11.00	6.27	9.12
Stems	9.45	8.97	7.88	8.43
Leaves	2.39	2.97	3.15	2.84

These data show that the carbohydrate-nitrogen ratio is not a constant as we think of a constant in mathematics or physics. In plants where great fluctuation occurs in their substratum throughout different parts of the day and different times in the season, this disparity is no positive evidence that such a ratio does not exist. Secondly, regardless of the exactness of the ratio, this much is true. when the carbohydrates are high the nitrogen compounds are relatively low, and vice versa. Figs. 9–11 show this reciprocal

condition in the roots, stems, and leaves in the various stages of development.

SEEDS

A tissue analysis of the seeds was made in order to discover what important compounds were present and the distribution of these compounds in the various fractions. This gives one a more comprehensive knowledge of the chemical constitution of the plant.

PREPARATION.—The seeds were freed from chaff and cleaned in a breeze until all red seeds were removed and only plump black ones remained. These uniform seeds were then ground in a mortar with the pestle by taking a few at a time. A known quantity (25 gm.) was weighed out in triplicate for alcoholic digestion, and the tissues were fractionated and the carbohydrates, the nitrogen compounds, and the phosphorus determined.

TABLE IV

SEEDS; SUMMARY OF TOTAL CONSTITUENTS

Material	A. blitoides		A. retroflexus		
H_2O Av. 3	.....	9.45	.....	8.61	.....
Total N	2.55	2.42	2.54	2.47	2.37
Total P	4.01	3.93	4.63	4.60	4.65
Total carbohydrates	48.27	48.43	47.22	47.03	47.37
Lipins	4.58	4.44	7.67	7.86	7.78
Ash (ignition)	3.50	3.68	4.27	4.19	4.14

DIFFERENT FORMS OF PHOSPHORUS.—After separating the seeds into fractions F_1, F_2, and F_3, the different phosphorus compounds were estimated in the three fractions. In each case the phosphorus was determined by the Pemberton-Kilgore method (10). The analysis of the different forms of phosphorus is given in table V, and the percentage ratio of these different forms to the total phosphorus is given in table VI. The inorganic phosphorus was estimated by the CHAPIN-POWICK method (4). The amounts obtained from fractions two and three (F_2+F_3) were combined and calculated as total inorganic phosphorus. The soluble organic phosphorus was obtained by subtracting the inorganic phosphorus of fraction two only from total phosphorus in the same fraction (F_2). The

phosphoprotein phosphorus was split off from the insoluble fraction (F_3) by digesting a weighed amount (1–3 gm.) with 1 per cent NaOH solution at 37–40° C. for 48 hours in an incubator, after which the solution was neutralized with acetic acid and made up to volume. The insoluble material was separated from the filtrate by the use of the centrifuge. The filtrate obtained was tested for phosphorus with magnesia mixture. The magnesium ammonium phosphate precipitated out by standing overnight was dissolved, and the phosphorus was reprecipitated as ammonium phosphomolybdate and then titrated against a standard alkali (8). The nucleoprotein phosphorus was estimated by the difference between the total phosphorus minus the phosphoprotein and the inorganic phosphorus in the two fractions (F_2+F_3). The lipin phosphorus was determined by taking an aliquot of the ether soluble fraction (F_1). The ether was first driven off on a steam bath before acid digestion and the percentage of this phosphorus was estimated in the same way.

TABLE V

DIFFERENT FORMS OF PHOSPHORUS (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	0.133	0.137	0.131	0.123	0.132
Lipin P	0.014	0.013	0.019	0.020	0.017
Soluble organic P	0.011	0.012	0.023	0.033	0.034
Phosphoprotein P	1.240	1.340	1.840	1.950	1.790
Nucleoprotein P	2.610	2.430	2.620	2.470	2.670
Total P	4.008	3.932	4.633	4.596	4.649

TABLE VI

RATIO OF DIFFERENT P TO TOTAL P (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	3.32	3.49	2.83	2.65	2.84
Lipin P	0.35	0.33	0.41	0.44	0.37
Soluble organic P	0.28	0.31	0.49	0.72	0.73
Phosphoprotein P	31.00	34.10	39.74	42.40	38.50
Nucleoprotein P	65.30	61.80	56.60	53.70	57.50
Total P	100.25	100.00	100.07	99.91	99.94

Tables V and VI show the percentage of the different forms of phosphorus. The data in these tables show that about 96 per cent

of the total phosphorus is in the organic combination in the seed, existing (perhaps) as phosphoprotein and nucleoprotein phosphorus. Both of these forms are insoluble in water, alcohol, ether, and alcohol-water solvents. The inorganic phosphorus is relatively low, and the writer believes that the figures given for inorganic phosphorus in table V are even too high, because the greater part of the inorganic phosphorus was obtained from the insoluble fraction F_3 (4). Moreover, there is no proof that the reagents used did not break down some of the organic phosphorus. The lipin phosphorus is very low, varying from 0.014 per cent in *A. blitoides* to 0.019 in *A. retroflexus*, calculated on dry weight basis. It is interesting to know that in all cases the different forms of phosphorus are relatively higher in *A. retroflexus* than the corresponding forms in *A. blitoides*.

TABLE VII

DIFFERENT NITROGEN COMPOUNDS IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total N	2.550	2.420	2.540	2.470	2.370
Nitrates NO_3	0.200	0.212	0.194	0.193	0.205
Amino N	0.096	0.095	0.089	0.090	0.090
Lipin N	0.027	0.027	0.031	0.032	0.033
Soluble proteins	2.260	2.270	2.660	2.790	2.890
Insoluble proteins	12.640	12.450	12.700	12.820	12.250
Total proteins	14.900	14.720	15.360	15.610	14.140

TABLE VIII

RATIO OF DIFFERENT N TO TOTAL N (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total insoluble N	83.20	82.00	80.40	79.00	77.00
Total soluble N	16.97	17.93	19.62	21.01	23.23
Total	100.17	99.93	100.02	100.07	100.23
Nitrate N	1.77	1.88	1.73	1.77	1.97
Lipin N	1.06	1.13	1.24	1.29	1.41
Amino N	4.68	4.65	3.50	3.66	3.84
Other soluble organic N	12.29	13.28	16.12	17.41	19.37

NITROGEN COMPOUNDS.—The distribution of nitrogen in the different fractions of the seeds is about the same as that of phosphorus. The insoluble nitrogen comprised 80–83 per cent of the total nitrogen (tables VII and VIII). The soluble fractions

contain only 17–20 per cent of the total, and most of it exists as organic nitrogen. The portion representing inorganic nitrogen is the nitrate nitrogen, which is relatively small. Calculated as nitrates (NO_3), the seeds contain 0.20 per cent, and this is equivalent to 1.80 per cent of the total nitrogen (tables VII and VIII). The lipin nitrogen is very small, only 0.027 per cent in *A. blitoides* and 0.032 per cent in *A. retroflexus*. These represent 1.10 and 1.31 per cent respectively of the total nitrogen content in these two seeds. In general a high percentage of insoluble phosphorus is accompanied by a high percentage of insoluble nitrogen, and a low percentage of soluble phosphorus by a low percentage of soluble nitrogen.

CARBOHYDRATES.—The polysaccharides are the predominating sugars in these seeds. *A. retroflexus* seeds contain 46 per cent and *A. blitoides* 47.75 per cent polysaccharides (on dry weight

TABLE IX

CARBOHYDRATES IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Lipin sugars	None	None	None	None	None
Reducing sugars	None	None	None	None	None
Non-reducing sugars	0.67	0.68	1.12	1.13	1.17
Polysaccharides	47.60	47.75	46.10	45.90	46.20
Total	48.27	48.43	47.22	47.03	47.37
	Ratio of different sugars to the total carbohydrates				
Non-reducing	1.38	1.40	2.37	2.40	2.47
Polysaccharides	98.65	98.60	97.60	97.60	97.60
Total	100.03	100.00	99.97	100.00	100.07

basis). If these sugars are calculated on the dry basis of the total sugars, the polysaccharides represent 97.60 and 98.60 per cent respectively in these two species. A striking contrast is seen on comparing the amount of polysaccharides in the green plant organs (figs. 6–8), which vary only slightly throughout the growing period, with that found in the seeds. In the growing period the highest percentage of polysaccharides was only 14.85, while that of the seeds was 47. In addition to this noticeable contrast, the soluble

sugars, both reducing and non-reducing, were comparatively high in the green plant organs (6–8 per cent), while those of the seeds are low (0.67–1.14 per cent).

LIPIN FRACTION.—The percentage of this fraction is 4.5 for *A. blitoides* and 7.78 for *A. retroflexus*. A closer examination of this fatlike substance shows that it contains phosphorus and nitrogen. The percentage of this nitrogen and phosphorus is very low (calculated on dry weight basis of whole sample). The presence of nitrogen and phosphorus indicates that the seeds contain phosphotides. The atomic ratio of nitrogen to phosphorus was determined by dividing the percentage by their respective atomic weights. The atomic ratio of N:P for *A. blitoides* is 1:2.3, and that for *A. retroflexus* is 1:2.6. This shows that other forms of phosphorus must be present than that existing in "ideal lecithin," which implies that the atomic ratio of N:P = 1:1 (**5, 13**).

In addition to lecithin, a phytosterol was present in the seeds. This could not be quantitatively determined by using animal cholesterol as a standard, because animal cholesterol (**17**) has a different tint from that of the plant. BUCHARD'S color reaction gives a deep blue color for animal cholesterol, while for the seeds it gives a yellowish green. The amount of phytosterol in these two species of seeds was compared. Assuming that the phytosterol in *A. blitoides* is unity, that in *A. retroflexus* is 2.8.

TABLE X

CONSTITUENTS OF LIPIN FRACTION (F_1) OF SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides			A. retroflexus		
Lipin, fats, etc. (wet basis)	4.15	4.07	4.23	7.02	7.18	7.09
Lipin (dry basis)	4.58	4.44	4.66	7.67	7.86	7.78
P in F_1	0.300	0.300	.....	0.240	0.260	0.220
P calculated in whole sample	0.014	0.014	.....	0.019	0.020	0.017
N in F_1	0.600	0.640	.....	0.402	0.400	0.420
N calculated in whole sample	0.027	0.027	.....	0.031	0.031	0.033

INORGANIC ELEMENTS.—The 1917 seeds of *Amaranthus retroflexus* were used for the estimation of the inorganic elements. The percentage of total ash given in table IV is 3.59 per cent for *A. blitoides* and 4.20 for *A. retroflexus*, but this is actually too low

for the inorganic elements present in the seeds, because the total phosphorus alone is 4.0 and 4.60 per cent respectively for the two species. This discrepancy is due perhaps to the loss of nitrates and part of the sodium, potassium, etc., in burning in the electric muffle. In the following analysis of the inorganic elements acid digestion (concentrated H_2SO_4+concentrated HNO_3) was used for the kations and alkaline fusion for the anions. The potassium was determined directly in the presence of all other elements except ammonia (NH_4—ion) and strong acid, as $K_2NaCO(NO_2)_6-\frac{1}{2}H_2O$ (11). Magnesium was estimated by the volumetric method as NH_4MgAsO_4 (7). The percentage of inorganic elements is as follows:

INORGANIC SALTS (*A retroflexus*); 1917 SEEDS

	Percentage	Percentage
Silica	0.42	0.40
$Al_2O_3+F_2O_3$	0.53	0.56
CaO	0.54	0.53
MgO	0.82	0.84
K_2O	0.38	0.35
Na_2O	no determination	
Cl	Trace	Trace
SO_3	0.34	0.33
P_2O_5	8.90	8.85
N_2O_3 (nitrates)	0.12	0.123
Total (not including Na_2O)	12.05	11.98

Discussion

In spite of the inaccuracy of BOUTIN'S method for the determination of the nitrates, his results are probably not far from correct. He stated his results in percentage of KNO_3 as shown in the second column of table XI. I have calculated these as percentage of NO_3, as shown in the third column. His percentages of nitrates are not far from those found by me for the stems (8.57 per cent) and branches (12.50 per cent) of *A. retroflexus*, July 8 collection, as shown in table II.

	Percentage KNO	Percentage equivalent in NO
A. retroflexus	22.77	13.92
A. blitum	11.68	7.17
A. ruber	16.0	9.82

Table XI shows the results of the analyses of the seeds by various authors. In the main there is fairly close agreement, but in some cases there are considerable discrepancies. The discrepancies can probably be explained by the different chemical methods used by the various authors and by the lack of uniformity in the different crops analyzed.

TABLE XI

COMPARISON OF SOME OF THE ANALYSES ON *Amaranthus* SEEDS

MATERIAL	PAMMEL AND DOX		HARDING AND EGGE			WOO	
			A. retroflexus				
	A. blitoides	A. retroflexus	20 mesh	72 mesh	Oven dry	A. blitoides	A. retroflexus
Condition of seeds			Non-	uniform		Matured uniform	Matured uniform
						Av. of 3	Av. of 3
H_2O				11.28	8.60	9.45	8.61
Lipins (fats)	Little*	Little*		7.92	8.46	4.56	7.77
Polysaccharides	Abundant	Abundant	39.77	40.98	44.83	47.68	47.21
Reducing sugars			Trace	Trace	Trace	None	None
Non-reducing sugars (sugar after inversion)			2.08	2.15	2.35	0.67	1.14
Nitrogen	1.88	2.49				2.48	2.46
Protein	11.75	15.59	18.57	19.13	20.93	14.81	15.03
Ash			4.33	4.46	4.88	3.59	4.20

* Microchemical test.

From the results of this study it would seem that *Amaranthus retroflexus*, and probably other species of the same genus, can bear, as they ordinarily do bear, large amounts of free nitrates without being forced out of reproduction into extreme vegetation. This genus apparently is endowed with a very high capacity for nitrate absorption, as well as for maintaining its full seed production power in the face of a great excess of free nitrates. In this respect it seems to differ from the tomato studied by KRAUS and KRAYBILL, and probably from many other plants. Considering all angiosperms, it is likely that, due to hereditary characters, there is a great range of ease with which plants can be forced to excessive vegetation by extreme nitrate supply within the plant. It is well known that a given level of fertility that will throw small grains into extreme straw production with deficiency of grain will give excellent grain production in corn. This may be due to the lower nitrate absorbing power of the corn, to its greater

photosynthetic activity to balance the nitrates absorbed, or to the higher carbohydrate-nitrogen ratio accompanying best grain production. Which of these three possibilities really determines the situation can only be answered by such studies as those made or suggested on the tomato by KRAUS and KRAYBILL, or studies of the type made in this paper. It is evident, however, that there is need of numerous studies of the carbohydrate-nitrogen ratio in plants, both in regard to the factors affecting this ratio and the effect of the ratio on plant characters. As was suggested in the review of the literature at the beginning of this article, such studies are likely to throw much light on other physiological features than vegetation and reproduction.

Summary

1. There is a large amount of nitrate in the organs of *A. retroflexus*. The stem and branches are the primary nitrate storage organs. The rate of nitrate absorption increases with the aging of the plant, perhaps partly being due to the development of the root system with numerous branching rootlets, increasing the radius of the feeding area from a few inches to 2 ft. or more.

2. This high capacity for nitrate absorption and storage must be an important factor in making *Amaranthus* a very successful competitor against cultivated plants, so effectively withdrawing as it does the nutrient element most commonly limiting plant production. It would be interesting to know how generally and to what degree weeds possess this power.

3. The carbohydrates and nitrogen compounds fluctuate throughout the growing period. The fluctuation of the carbohydrates is in the reverse order of the nitrogen compounds. This inverse ratio is not a truly mathematical constant, but in general when the carbohydrates are high the nitrogen compounds are low, and vice versa. As the nitrate nitrogen composes more than 50 per cent in the stems and branches, there is a possibility that nitrates have some modifying effects on this reciprocal relationship. This inverse ratio is due partly to the synthesis of protein, chlorophyll, phospholipin, and other organic nitrogen compounds at the expense of the soluble carbohydrates.

4. Tissue analysis of the seeds shows the distribution of different forms of phosphorus in the various fractions. The organic phosphorus, which consists chiefly of phosphoprotein and nucleoprotein phosphorus, is high, and that of the inorganic form is low.

5. The distribution of nitrogen in seeds is in the same order as that of the phosphorus. The insoluble portion contains 80–83 per cent of the total. The soluble part varies from 17 to 20 per cent, most of which is in the organic form. The inorganic form is represented by the nitrate nitrogen.

6. The predominating sugars in the seeds are the polysaccharides. These compose nearly one-half of the total dry weight of the seeds. In both *A. retroflexus* and *A. blitoides* there is absence of lipin sugars in F_1 and reducing sugars in F_2. Only a small amount of non-reducing sugars was present in the two varieties.

7. The presence of nitrogen and phosphorus in the lipin fraction indicates that the seeds contain phosphatides. Phytosterol was also present. By comparison, *A. retroflexus* has 2.8 times as much as *A. blitoides*.

I am indebted to Drs. Wm. Crocker, S. H. Eckerson, and F. C. Koch for their kind advice and aid during the progress of the work.

University of Chicago

LITERATURE CITED

1. Boutin, A., Sur la présence d'une proportion considérable de nitre dans l'*Amaranthus blitum*. Compt. Rend. **76**:413–417. 1873.
2. ———, Sur le présence d'une proportion considérable de nitre dans deux variétés d'*Amaranthus*. Compt. Rend. **78**:261–262. 1874.
3. Brosset, —, Sur quelques passages de Stan. Bell, d'où l'on peut conclure que l'*Amaranthus blitum* est cultivé en circassie pour le nitre qu'il contient. Compt. Rend. **79**:1274. 1874.
4. Chapin, R. M., and Powich, W. C., An improved method of the estimation of inorganic phosphoric acid in certain tissues and food products. Jour. Biol. Chem. **20**:97–114. 1915.
5. Czapek, F., Biochemie der Pflanzen. 2d ed. **1**:763–773. 1913.
6. Fischer, H., Zur Frage der Kohlensame-Ernährung der Pflanzen. Gartenflora **65**:232–237. 1916.
7. Fox, Paul J., Titration of calcium and magnesium in the same solution. Jour. Ind. Eng. Chem. **5**:910–913. 1913.

8. HARDING, E. P., and EGGE, W. A., A proximate analysis of seed of common pigweed, *Amaranthus retroflexus* L. Jour. Ind. Eng. Chem. **10**:529–530. 1918.
9. HEDLUND, T., Über die Möglichkeit, von der Ausbildung des Weizens in Herbst auf die Winterfestigheit der verschiedenen sorten zu schliessen. Rev. Bot. Centralbl. **135**:222–224. 1917.
10. HIBBARD, P. L., A study of the Pemberton-Kilgare method for determination of phosphoric acid. Jour. Ind. Eng. Chem. **5**:998–1009. 1913.
11. KOCH, W., Methods for quantitative chemical analysis of animal tissue. Jour. Amer. Chem. Soc. **31**:1329–1364. 1909.
12. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Exp. Sta. Bull. 149. pp. 90. 1918.
13. MACCLEAN, HUGH, Lecithin and allied substances, the lipines. Monograph Biochemistry, pp. vii–206. 1908.
14. Official and provisional methods of analysis. Association of Official Agricultural Chemists. Bull. 107. Bur. Chem., U.S. 1907.
15. PAMMEL, H. L., and DOX, A. W., The protein contents and microchemical tests of the seeds of some common Iowa weeds. Proc. Iowa Acad. Sci. **22**:527–532. 1917.
16. RIBERA, U., Über die Ursache des Lagems beim Weizen. Internat. Agric. Techn. Rundschau **7**:524–525. 1916.
17. WESTON, P. G., Colorimetric methods for determining serum cholesterol. Jour. Biol. Chem. **28**:383–387. 1917.

STAMINATE STROBILUS OF TAXUS CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 255

A. W. DUPLER

(WITH PLATES XXIV–XXVI AND TWENTY-TWO FIGURES)

Introduction

In a previous paper (8) the writer described the gametophytes of *Taxus canadensis* Marsh., with the statement that other phases of the morphology would be treated in later papers. In this paper the staminate structures with respect to development and vascular anatomy are described. The lack of detailed information concerning these structures has seemed to the writer sufficient justification for the investigation here reported. In view of the generally recognized conservative character of the staminate structures in conifers, it seems that a more extended investigation of them, in the group as a whole, would be worth while. The description of the ovulate structures will be given in another paper.

The general statement in the previous paper as to material and methods will also apply here. The writer is under obligations to Professor W. L. EIKENBERRY, of the University of Kansas, for some material collected in northern Illinois a number of years ago. Acknowledgments are also due Professors JOHN M. COULTER and C. J. CHAMBERLAIN, under whose direction the study of *Taxus canadensis* was begun.

Historical

While the male gametophyte and its attendant features have received considerable attention, apart from the general more obvious features very little is found in the literature dealing with *Taxus* as to the morphology of the staminate strobilus itself. The earlier workers who studied the staminate structures of conifers were concerned largely in attempts to interpret them in terms of the angiosperm flower, naturally leading to confusion as to the true nature of the structures. These earlier views have been summarized by VON MOHL (26) in perhaps one of the most important

of the early papers dealing with the "male flowers" of conifers, and to which we are indebted for part of the following statement regarding this early interpretation of the staminate strobilus of *Taxus*.

LINNAEUS (16) regarded the entire strobilus as a single flower, with the stamens in a cylinder, the perianth lacking and replaced by bud scales. JUSSIEU (13) held that the strobilus was a monadelphous flower; while LINDLEY (15) considered the strobilus as a true cone with naked monadelphous flowers, each sporophyll representing a flower. RICHARD (19) went still farther, with the rather unique view that there were 5–8 flowers under each scale to which the stalk of the flower is attached on the underside. According to this view the pollen sac represented a "flower," and he had a similar interpretation for the sporophylls of *Thuja* and *Juniperus*. ZUCCARINI (28), regarding the reproductive structures as modified portions of the stem, comparable with the phylloclads of *Phyllocladus*, described the anther of *Taxus* as 7–8-lobed around the tip of a central column. He considered that *Taxus* has the most complete male flower in conifers, in other forms the anther folds growing on only one side of the central column, the other side growing out into a scale. VON MOHL opposes the idea of the stem character of the "flower" of conifers, and objects to the view that the "anthers" of other conifers have been derived from such a structure as that of *Taxus*, because "we have yet no certain data with which we can determine with certainty whether the anther of *Taxus* arises from one leaf or from a whorl of leaves."

As compared with our present ideas these early views are rather strange, having largely only a historical interest, with very little bearing on the real morphology of the structures concerned. Considerably later STRASBURGER (22) made some observations on *T. baccata*, describing the spiral arrangement of the scales of the strobilus and the grosser features of the development of the sporophylls. He held that the peltate stamen of *Taxus* represents the "extreme form of stamen," and found that it begins as a rounded knob about the first of August, becomes lobed by lateral swellings due to internal growth, and that pollen mother cells form in these lateral swellings and produce pollen by tetrad divisions. He also describes the pollen region as separated from the epidermis by two

layers of irregular cells, and states that dehiscence is accomplished by the rupture of cells at the base and sides of the pollen sac. CHAMBERLAIN (3) describes the microsporangium of *T. canadensis* at the mother cell stage (October 1, 1897), at which time the nuclei are still rather small in comparison with the size of the cell, the tapetum being sharply differentiated, and its cells showing no tendency to plasmolyze like the cells of the sporangium wall. PILGER (18) describes the general external features, largely from the taxonomic viewpoint, speaking of the "flower" as consisting only of sporophylls surrounded at the base by a scale envelope which completely incloses the flower in the bud state. He regards the "leafy structure" of the anther, which is yet to be recognized in *Torreya* and *Cephalotaxus*, as being "entirely lost" in *Taxus*.

In the related forms the staminate structures of *Torreya* have been described in a general way by PILGER, based on *T. nucifera;* in more detail by Miss ROBERTSON (20) for *T. californica; and* by COULTER and LAND (5) for *T. taxifolia*. In *Cephalotaxus* some of the features of the spermatogenesis have been described by STRASBURGER (23), and by ARNOLDI (1). STRASBURGER (24) pointed out that the pollen grain divides in the sporangium before shedding; LAWSON (14) also confirms this in *C. drupacea;* and WORSDELL (27) gives a description of the general features of the "male flower," based on *C. Fortunei*, comparing it with those of other forms (*Phyllocladus* and *Ginkgo*), especially in the sporophyll features.

Strobili buds

In the axils of the leaves of the shoot of a given season 3 types of structures are produced: (1) the vegetative buds from which develop the lateral leafy shoots of the next season; (2) the young staminate structures, maturing the next season; and (3) the ovule-bearing structures, also maturing the next season. During the first season all of these structures are in bud form, the staminate buds during the latter part of the summer and winter being more globular than the other two kinds, which are so nearly alike in external appearance as to make their distinction uncertain except by very careful examination.

The rudiments of these structures begin to develop very soon after the beginning of growth of the terminal bud in the spring, the

FIGS. 1–6.—Longitudinal sections of young buds: fig. 1, young vegetative shoot with bud rudiment in axil of young leaf; fig. 2, vegetative bud with conical apex; fig. 3, staminate bud with broadened apex; fig. 4, ovulate bud, showing vegetative tip (to left) and rudiment of ovulate strobilus in axil of scale; fig. 5, young staminate strobilus, showing primordia of stamens and tip of axis; fig. 6, young staminate strobilus with primordia of sporophylls, axis apex not evident; ×36.

rudiments appearing as conical projections in the axils of young leaves (fig. 1). By the middle of June these axillary structures have

attained a length averaging about 1.5 mm., consisting of the main axis surrounded by compactly arranged scales. In this early stage one cannot distinguish these structures from one another, either by external appearance or in section. Early in July, however, one can recognize in median longitudinal sections the beginning of the differentiation which is now taking place, the apex of the vegetative bud remaining conical (fig. 2), as is characteristic of the vegetative stem tip (fig. 1), the apex of the staminate structure becoming broadened (fig. 3), while the ovule-bearing structure is recognizable by the rudiment of the ovulate strobilus appearing in the axil of one of the scales near the tip of the primary shoot (fig. 4). All 3 kinds of buds may occur on the same shoot; in fact, this is the usual occurrence, with the staminate buds generally the more numerous, the vegetative buds nearest the tip, and one to several ovulate buds a short distance below the vegetative ones, the staminate buds occupying the older portion of the shoot.

The buds arise only on the current season's growth, and in case of the staminate structures always mature the next season. No cases were observed in which staminate strobili were produced on older growth, nor were any cases found in which the buds remain dormant for a time and then mature. Miss ROBERTSON, in her study of *Torreya californica* from trees growing in England, found that while the staminate strobili are formed in the axils of the leaves of a current season, they may remain dormant for as long as 3 years. In *Taxus* buds may be found on older growth, but they are either dormant vegetative buds or persisting primary shoots of the ovuliferous structures of a former season, as will be described more fully in the paper dealing with these structures.

Sporophylls

PRIMORDIA

The broadened apex (fig. 3) is the first indication of the true nature of the staminate strobilus bud, and can be recognized first about July 1. STRASBURGER (22) was able to recognize the staminate structure of *T. baccata* about August 1, and in *Torreya taxifolia* COULTER and LAND (5) first observed the staminate buds in July,

but the primordia of the sporophylls did not begin to appear until August. The greater meristematic activity in some regions of this rounded apex than in others marks the position of the primordia of the sporophylls. These soon become rounded lobes above the general surface (figs. 5, 6). The nature of the growth of the primordium would indicate that it arises from a group of meristematic cells rather than from a definite initial; at least no defined sporophyll initial could be recognized.

The sporophylls are probably spirally arranged, although this is somewhat indefinite, and indications were found in a few cases that they may arise in acropetal succession (fig. 5); but if this is the case it is very soon obscured in the uniform development of the primordia as the sporophylls develop, no trace of the axis apex being recognizable after the very early beginnings of the sporophylls. The early development of the primordium is uniform in all directions from its central axis, at least until the differentiation of the archesporial initials takes place. The strobilus in this stage shows a series of rounded sporophyll primordia (figs. 5, 6, 23). The later development of the sporophyll is so intimately bound up with the development of the sporangia as to best be described in connection with them. In fact, the development of the sporangia determines the shape and character of the sporophyll, as aside from the sporangia the sporophyll consists of practically nothing excepting the short central axis and the epidermis.

MICROSPORANGIUM

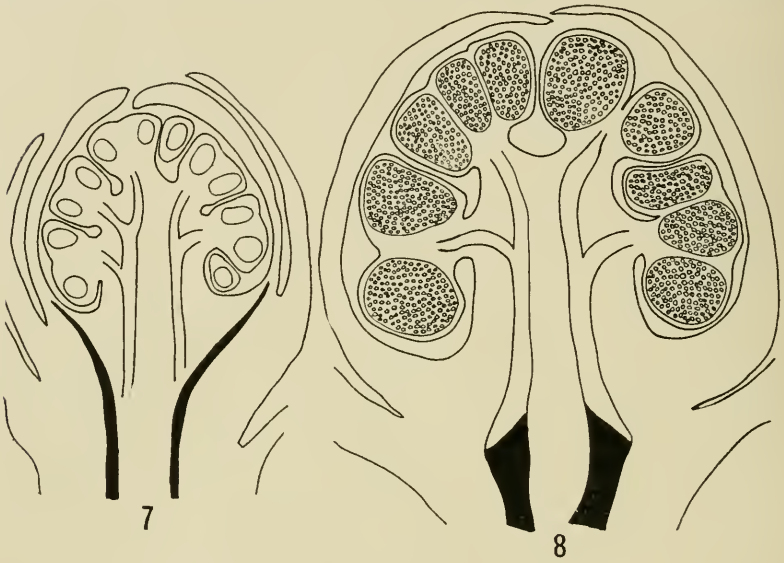
ARCHESPORIAL INITIALS.—HOFMEISTER (11) seems to have been the first to publish with reference to the microsporangium of conifers, reporting the spore mother cell stage as being reached in *Pinus maritima* in November. GOEBEL (9) traced the archesporium of *Pinus* to a single hypodermal cell, and claimed a similar origin for the archesporium of *Thuja*. His most important observation on this point was that the development of the microsporangium is like that of the eusporangiate ferns. COKER (4) in *Taxodium distichum*, and NICHOLS (17) in *Juniperus communis* var. *depressa*, also found a hypodermal origin of the archesporium, in the latter case consisting of "a plate of radially elongated cells, 4–6 in

number, when viewed in longitudinal section." COULTER and LAND, interpreting the structures in abortive sporangia, conclude that in *Torreya taxifolia* there is "a single hypodermal archesporial cell." Miss ROBERTSON did not get the origin of the sporogenous tissue in *T. californica*.

In *Taxus canadensis* the development follows the usual eusporangiate method, the 4–8 (usually 5–7) archesporial initials arising from the hypodermal layer of the sporophyll primordium while this structure is yet quite small (fig. 23), being uniformly distributed along its margin, and, dividing by periclinal walls, form the primary wall cell and the primary sporogenous cell (figs. 27–29), as in *Torreya taxifolia* (5) and most other forms. These initials are first to be recognized by the size of the cells and of their nuclei (figs. 23–26). One initial cell seems to be the rule, although cases were found in which the archesporium consists of 2 cells (fig. 26).

SPOROGENOUS TISSUE.—The primary sporogenous cell or cells soon divide periclinally (fig. 30) or anticlinally before or after the division of the primary wall cell, and by successive divisions the mass of the sporogenous cells is increased (figs. 31–35), the formation and growth of which result in the lobed peltate structure of the sporophyll, the sporangia being uniformly distributed around the central axis which continues the very short stalk of the sporophyll. As the tissue increases there is a corresponding growth of the epidermis and the sporangium wall (to be described later), the completion of which results in the separation of the sporogenous tissue from the other portion of the sporophyll (fig. 34). The tapetum is differentiated from the peripheral layer, and the remaining sporogenous mass increases in amount until the mother cell stage is reached early in October, as described by CHAMBERLAIN (3) and the writer (8). This has been given (6), and even quite recently (7), as the winter condition of the microsporangium, and has frequently been quoted by writers. As the author has already pointed out (8), microspore formation takes place during the early part of October, collections covering a number of years and from several localities in the northern United States bearing out the statement that the microspore is the winter condition of *Taxus canadensis*. STRASBURGER (25) found microspore formation in *T. baccata* taking place

in February in 1904, during unusually warm weather, indicating that in this form the sporogenous tissue remains in the mother cell stage until spring. It would be of interest to know the behavior in the extreme northern part of the range of *T. canadensis*, as it is possible that in regions farther to the north the microspore stage might not be reached before winter. The microspore mother cell stage is the winter condition of *Torreya californica* (20) in England



FIGS. 7, 8.—Median longitudinal sections of older strobili: fig. 7, at time of completion of sporangium wall, showing oval areas of young sporogenous tissue; vascular tissue of axis and upper scales, shown in black, embryonic vascular tissue of upper portion in outline; fig. 8, winter condition of strobilus, showing globular character of bud and microspores; vascular tissue as in preceding figure; ×36.

and of *T. taxifolia* (5) in Florida. During this development the strobilus has grown considerably in size (cf. figs. 7 and 8), becoming more pronouncedly globular, and it remains in this condition until the renewed growth of spring takes place.

No cases of abortive sporangia were found, and it seems a safe assumption that a sporangium develops from each initial or initial group. The adult sporangia show some variation in size, but not enough to indicate any tendency to abortion of any of them. This

is in marked contrast with the behavior in the related *Torreya*, in which a resin cavity results from the abortion of some of the potentially sporogenous tissue of the sporophyll, the abortion beginning at the primary sporogenous cell stage, as pointed out by COULTER and LAND for *T. taxifolia*. This results in the sporangia occurring on only one side of the otherwise peltate sporophyll. Miss ROBERTSON also finds that normally there are 4 sporangia on the side of the sporophyll of *T. californica* and a resin cavity on the other side, but that the strobilus axis sometimes terminates in a radially symmetrical sporophyll, like that of *Taxus*, with 6 or 7 mature sporangia. Whether a resin cavity is present in such a sporophyll is not stated, the inference being that most or all of the sporangium initials reached maturity. A similar abortion of sporangia, in the formation of mucilage cavities, is indicated by Miss STARR'S (21) work on *Ginkgo biloba*. COULTER and LAND find in *Pinus Laricio* resin cavities related to sporangia, exactly as are the lateral sporangia to the two middle ones in *Torreya*, and say "there is evident a tendency to reduce the number of sporangia by abortion, a reduction that has proceeded farther in *Pinus* than in *Torreya*, and in the latter farther than in *Taxus*." It seems that when resin or mucilage cavities are present in the sporophyll the sporangium initials are involved, and when absent these initials may all function normally, as in *Taxus*. Whether this can be made as a general statement for all forms with resin cavities in the sporophyll must wait for more extensive work on other forms. *Cephalotaxus* has a sporophyll similar in general appearance to that of *Torreya*, but it is not known whether any abortion takes place.

SPORANGIUM WALL.—The initial development of the wall is from the primary wall cell, which by a periclinal division forms a tier of 2 cells. As the sporogenous tissue develops, these wall cells divide anticlinally (fig. 31), increasing the extent of the wall layers. Only a portion of the wall is derived from the primary wall cell, however, as other cells abutting the young sporogenous tissue divide periclinally and add to the wall, first on the outer side (figs. 31–33) and then on the inner side as well (fig. 34), thus completely enveloping the young sporogenous tissue. The wall usually consists of 2 layers of cells, although 3 or even more layers may be present,

especially at the angles formed by the mutual pressure of the sporangial lobes.

As the sporogenous tissue increases in size there is pressure upon the wall cells, and they become flattened and extended, so that at the time of their maximum development they are broad thin plates. By the time of spore formation they are usually quite flattened (fig. 36), and during the further growth of the spores become more or less disorganized, so that by the time the spores have reached maturity, just before shedding, the wall has become a very thin layer abutting the epidermis, which has now become, in effect, the functional sporangium wall.

TAPETUM.—At the time when the sporangium wall is completed the sporogenous tissue inclosed within it is uniform in appearance (fig. 34). Soon, however, the peripheral layer of this tissue becomes differentiated as a tapetum (fig. 35), thus originating from the sporogenous tissue and not from the inner layer of the sporangium wall, as in some forms. The tapetum, however, has its chief significance from a physiological standpoint, being generally regarded as a nutritive layer, its origin seeming to be of little morphological significance. The tapetal cells are usually uninucleate, but not infrequently are binucleate (figs. 35–36). The tapetum is quite distinct during later phases of the development of the sporogenous tissue, is sharply differentiated at the spore mother cell stage, as pointed out by CHAMBERLAIN (3), and remains distinct during the early winter (fig. 36). With the growth of the microspores in the spring it becomes less and less prominent, until near pollination it consists of only a very thin layer of disorganized material surrounding the spore mass.

EPIDERMIS

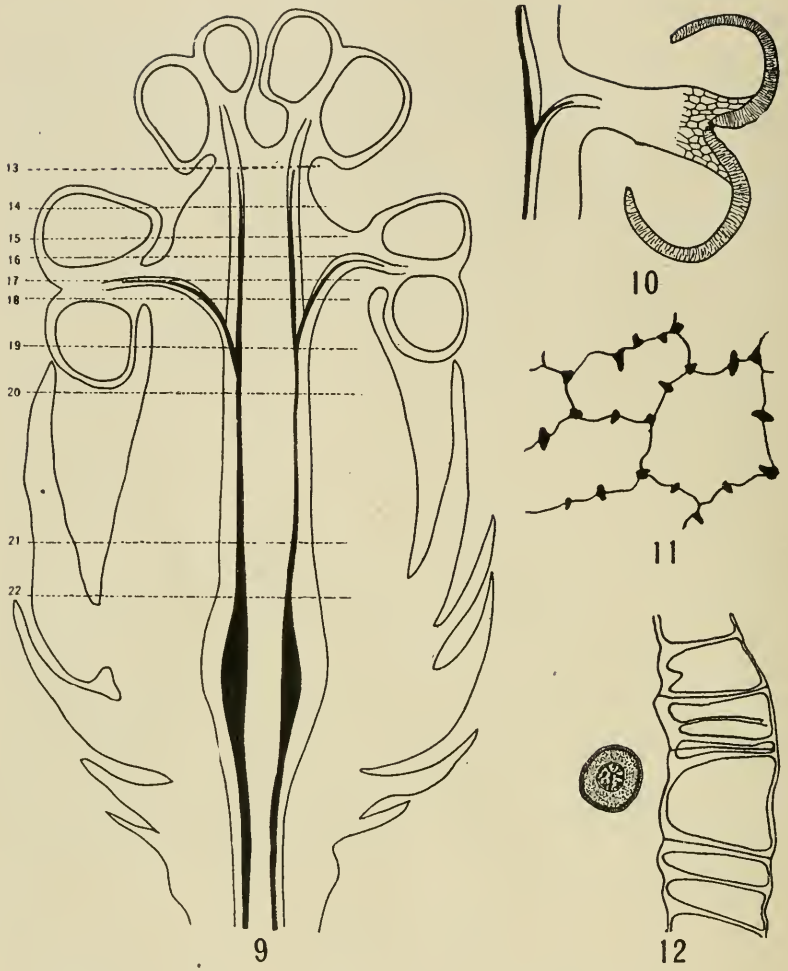
From the beginning of the primordium to the mature sporophyll the epidermis remains as a distinct layer, the sporangium developing from hypodermal tissues, as already stated. During the early growth of the sporophyll the epidermis is meristematic throughout, dividing anticlinally (fig. 24), its surface area thus keeping pace with the increase in the mass of the sporogenous tissue. An occasional periclinal division results in the epidermis becoming 2 cells thick at

some points. The meristematic ability, however, soon becomes limited to the base of the sporangium, the epidermal cells of the remainder of the sporophyll becoming larger and with less dense contents, the cells at the base remaining isodiametric and rich in cytoplasm (figs. 32–34). As the sporangium increases in size, causing a more pronounced lobing of the sporophyll, the necessary increase in epidermal surface is effected by the enlargement of the non-meristematic cells and the addition to them of cells from the basal meristematic region. The enlarged cells become filled with an amorphous substance and the walls become thicker.

By the time the sporangia are mature the epidermis has become the functional wall of the sporangium, owing to the practical disintegration of the true sporangium wall. At maturity the epidermal cells are devoid of contents and have the markings characteristic of the walls of many sporangia (figs. 11–12), these thickenings exercising a hygroscopic effect, useful in the liberation of the spores. Jeffrey (12) regards this thickening of the epidermal cells of the sporophyll, in a mechanical dehiscing device, as the result of the invasion of the epidermis by mechanical tissues of fibrovascular origin. There are no indications in *Taxus* of mechanical elements elsewhere in the sporophyll. Coulter and Land found numerous stomata in the epidermis of *Torreya*. In *Taxus canadensis* there is a single stoma on a sporophyll, at the center of the peltate disk, occupying the bottom of the depression caused by the enlarged sporangia (fig. 10). Goebel (10) shows a similar situation in *T. baccata*.

Mature strobilus

The scales at the base of the strobilus are small and decussate, increasing in size and becoming spiral in arrangement above, the uppermost ones being considerably larger than the lower ones, and function as bud scales in the immature condition of the strobilus. The scales are brownish in color, with heavily cutinized outer epidermal walls, especially on the abaxial surface, the stomata occurring only on the inner surface (fig. 37), reversing the condition on the vegetative leaves of the plant, where the stomata occur only on the lower (abaxial) surface. The midrib is marked by the



FIGS. 9–12.—Fig. 9, median longitudinal section of mature strobilus just before pollen shedding, showing "elongating region" of axis and 4 sporophylls; xylem of bundle black; note that xylem becomes centrally placed in upper portions and does not extend as far into stalks as phloem; numbers at left (13–22) indicate approximately levels of cross-sections of strobilus shown in figs. 13–22; fig. 10, median section of open sporophyll, showing elongated stalk, open sporangia, and solitary stoma in center of depression of disk; fig. 11, tangential section of mature epidermis, showing mechanical thickenings on walls; fig. 12, cross-section of mature epidermis, with microspore, at time of shedding; figs. 9, 10, ×36; figs. 11, 12, ×475.

vascular bundle, when present, and occasionally by sclerenchyma-like cells along the outer margin of the midrib. In the young strobilus the mesophyll of the scale is compact, but as the strobilus matures large air spaces develop. In addition to the solitary stoma found on the sporophyll, stomata occur on the strobilus axis between the bases of the sporophylls with rather surprising frequency, being found only on this portion of the axis and not on the portion between the upper scales and the lower sporophylls. While the functional character of these stomata might be open to question, owing to their position rather than to their structure, their chief interest probably lies in their morphological significance as hereditary structures from a more highly vegetative ancestral strobilus.

During the autumn, winter, and early spring the strobilus has the appearance of a globular "bud," the stamens being surrounded by the uppermost scales (fig. 7). The axis between the upper scales and the bases of the lower sporophylls is very short and remains so until a few days before maturity, during the latter part of April in central Pennsylvania, at which time there is a rapid enlargement and elongation of this portion of the strobilus, the effect being to push the sporophyll-bearing portion beyond the scales (fig. 9). A similar elongation of this region is reported for *Torreya californica* (20). COULTER and LAND described an enlarged pith region in the axis of the strobilus in *T. taxifolia*, which the authors suggest may be "an important storage region for the strobilus." No such enlarged region was found in *T. canadensis*. In addition to the elongation of this portion of the strobilus axis there is also an elongation of the stalk of the sporophyll (cf. figs. 9 and 10), resulting in the separation of the sporophylls from one another.

The sporangia do not hang freely from the underside of the disk, but are fused with the stalk on the inner side (fig. 9), and laterally are separated from one another only by thin partitions, the external furrows between the sporangia not extending all the way to the center, the sporophyll and sporangia thus constituting a very compact structure. RICHARD (19), STRASBURGER (22), and GOEBEL (10) gave accounts of the dehiscence of the sporangium of *T. baccata*, in which they pointed out the rupture of the sporangia at the base and the umbrella-like movement of the epidermal wall.

The process is the same in *T. canadensis*, the breaking of the thin-walled epidermal cells at the base of the sporangium in a circle around the base of the stalk, the rupture of some of the cells at the side of the sporangium, and the hygroscopic rôle of the thickened epidermal cells resulting in the wall of the sporangia spreading out in umbrella form, the thin partitions between the several sporangia also being broken in the process.

When young the strobili rudiments are erect in the axils of the leaves, but as they develop they become oriented in such a way as to hang pendent on the lower side of the shoot, the fertile portion of the strobilus being directed downward. GOEBEL (10) regards the position and the method of dehiscence such as to secure the most advantageous distribution of the pollen.

Vascular features

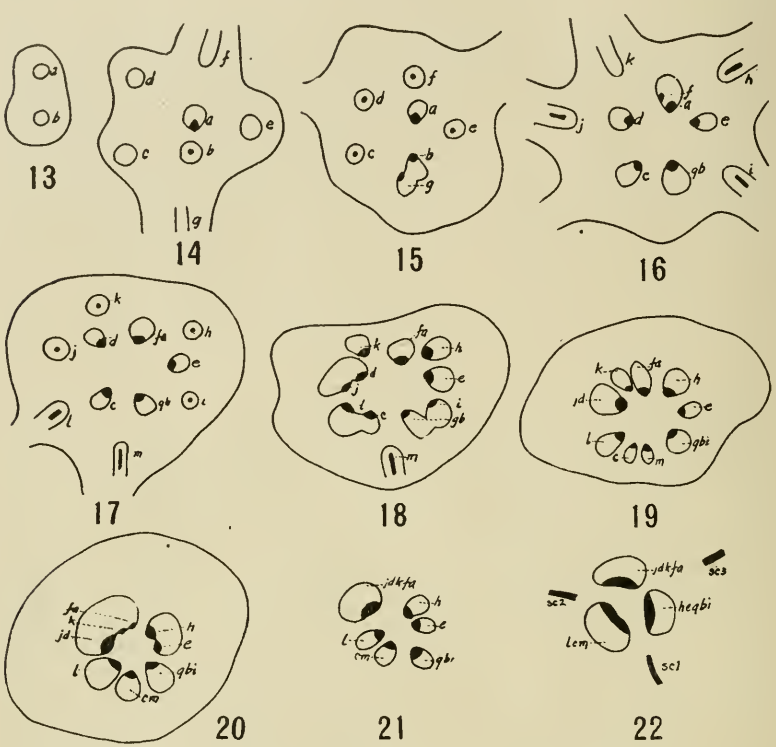
Since the reproductive organs, and especially the staminate structures, are regarded as among the most conservative of plant organs, a consideration of the vascular anatomy of the staminate strobilus is not without interest. While the ovulate strobili of conifers have been the subject of considerable investigation and discussion, in their vascular as well as in other features, the staminate strobili have not received much attention in their vascular anatomy, probably not as much as they deserve in view of the conservative nature generally assigned to them on other grounds. The only reference to this feature of *Taxus* is by STRASBURGER (22), who gave the arrangement of the scales of *T. baccata* and states that each stamen contains a bundle which passes into the stalk.

Like any other branch, the strobilus axis receives 2 bundles from the cylinder of the leafy shoot. These are semicircular in outline, and by meeting at their edges soon form a closed cylinder, broken here and there by the gaps formed by the weak bundle traces of the scales. In the lower portion of the strobilus, where the scales are small and decussate, the small traces often end in the cortex and do not reach the scale itself. The traces for the upper scales are better developed and extend for some distance into the midrib of the scale, especially in the 2 or 3 uppermost scales. Although the axis cylinder, as well as the cortical portion of the scale traces, are

collateral endarch, in their terminal portions they contain not only centripetal xylem, but are also accompanied by transfusion tissue which may be both dorsal and lateral to the xylem elements (fig. 38).

At the level of the uppermost scales the cylinder consists of 3 or 4 large bundles (figs. 22, 48) which extend into the fertile portion of the strobilus, where they branch, giving off finally a branch to each sporophyll, the bundle extending a little way into the base of the sporophyll stalk. In a young strobilus these bundles are represented only by elongated thin-walled elements, evidently procambium strands, which traverse the region between the base of the sporophylls and the level of the upper scales (figs. 7, 8). These strands remain in this embryonic condition until near maturity, when they elongate and take on their vascular features in connection with the growth of the "elongating region" of the strobilus axis. In this "elongating region" the pith becomes larger in diameter than in the lower portion of the strobilus, but shows no evidence of being in any way a storage region; in fact, there would be little use of a storage tissue at this stage in the development of the strobilus. The several large bundles of the strobilus axis extend for some distance into the "elongating region" and then give off branches to the various sporophylls, each of the large bundles supplying several sporophylls in this way (figs. 13–22). Some of the branches may unite and then separate (see the behavior of bundles *h* and *e*, and also of *l*, *c*, and *m*, in figs. 18–22), although usually the bundles pass rather directly to the base of the sporophyll (figs. 14–17, 44–47). Throughout the entire axis there is relatively a stronger development of the phloem than of the xylem, the latter forming a narrower zone than the former (fig. 48). Both xylem and phloem reach their greatest development near the level of the upper scales (figs. 9, 22), above this the xylem forming only a very narrow portion of the bundle. Throughout the strobilus the xylem consists of spirally thickened tracheids with bordered pits, the tracheids being rather short, however, although in the elongated region of the axis they are somewhat longer than at a lower level and the bordered pits are fewer in number. The phloem of the portion above the scales shows very little of the pitting present at a lower level, consisting

of elongated cells similar to those of the younger condition of the strobilus. Occasionally the xylem extends a short distance into the stalk of the sporophyll, the bundle here, however, usually consisting



FIGS. 13–22.—Cross-sections of mature strobilus at approximately levels indicated by numbers to left of strobilus shown in fig. 9; branches of bundles to various sporophylls indicated by *a*, *b*, *c*, etc., xylem indicated by black; bundles *a* and *b* supply terminal sporophylls; union of bundles indicated by combining letters, as *jd* in figs. 17–19; fig. 19 shows complete cylinder below lowermost sporophyll; in fig. 18 *l* and *c* united, in fig. 19 separated, in fig. 20 *c* and *m* united, and in fig. 22 *lcm* one of the 3 large strands from sterile portion of axis; fig. 22 also shows traces to 3 uppermost scales, *sc1*, *sc2*, and *sc3;* note concentric character of terminal portions, as in *c*, *d*, and *e*, in fig. 15; ×36.

only of the phloem portion (fig. 39), the xylem usually ending within the cortex of the axis.

The bundles of this region are collateral endarch in the lower portions. In the upper portions, however, the bundles frequently show centripetal xylem (figs. 40, 44), giving mesarch bundles, and

in some cases the smaller xylem elements are on the outside of the bundle, indicating a possibility of exarch structure (fig. 42). In addition, the xylem elements, in the terminal portions of the bundles, become more and more placed toward the center of the bundle, giving virtually a concentric bundle of a few xylem cells surrounded by the phloem portion of the bundle (fig. 41). No transfusion tissue was found elsewhere than in the scales.

Discussion

Perhaps the two most important features of the staminate strobilus of *Taxus* are the peltate sporophylls and the character of the vascular bundles of the scale and sporophylls. The peltate (epaulet) type of stamen occurred among the Paleozoic Cycadofilicales, in the *Crossotheca* forms, but the sporangia were bilocular and dehisced by a longitudinal slit along the adaxial face, the bilocular character being different from that of the modern gymnosperms. Peltate stamens are not known in Bennettitales, and none occur in the Cycadales. The peltate stamen has been carried forward to modern plants through the Cordaitalean line, in all probability, although so far as is known the stamens in the Cordaitales bore terminal erect sporangia. As COULTER and CHAMBERLAIN state, however, "it cannot be supposed that the stamens of so great a group were uniform in type," and it is very possible that peltate stamens occurred there also. The sporophyll of *Ginkgo* gives a suggestion of the peltate type of stamen, in occasionally having more than 2 sporangia, in the regular occurrence of more than 2 sporangia in fossil forms, and in the possibility, pointed out by Miss STARR, that the mucilage cavity replaces abortive sporangia. Among Coniferales there is a suggestion of the peltate stamen in the Araucarineae, and stamens of true peltate form occur in such forms as *Widdringtonia*, *Torreya*, and *Taxus*. In *Torreya* the true peltate character is generally obscured in the adult sporophyll owing to the development of the resin cavity from 3 of the 7 sporangium beginnings. Hence it is seen that peltate stamens, in one form or another, are scattered from Cycadofilicales to modern conifers, and there is no necessity of regarding such a sporophyll as that of *Taxus* as being of recent evolution. Assuming peltate

sporophylls in Cordaitales as probable, their continuation in Ginkgoales and Coniferales is quite possible, abortion of some of the sporangia in the formation of mucilage or resin cavities, in such forms as *Ginkgo* and *Torreya*, obscuring their true nature, but showing the true peltate character when all of the sporangia develop, as in *Taxus*. WORSDELL, following the view put forward by CELAKOVSKY (2), considers the peltate sporophyll of *Taxus* to have been derived from such a form as occurs in the Cordaitales, where the pollen sacs are "erect and terminal on the radial sporophyll," through such forms as found in *Cephalotaxus* and *Torreya*, where the pollen sacs are "sub-terminal and pendulous, owing to a slight prolongation of the axis of the sporophyll, between and beyond the sacs, in a small protuberance," this condition being intermediate between the Cordaitales situation and *Taxus*, "where the extended terminal portion has become enlarged and flattened out into a very distinct peltate structure." "*Taxus* thus represents an advance from the earlier types of *Cephalotaxus*, *Ginkgo*, etc., toward the subpeltate dorsiventral type of sporophyll of the true Coniferae." One must question the necessity of such an explanation for either the peltate sporophyll of the taxads or the dorsiventral one of most conifers, in view of the historical occurrence of both of these types in forms more primitive than even the Cordaitales.

The significant features of the vascular anatomy of the strobilus are the mesarch character of the terminal portion of the scale bundles, as well as the appearance of centripetal xylem in the terminal portion of the sporophyll bundle, where the bundle is not only mesarch at times, but may also be exarch and concentric. This indicates the very conservative nature of the staminate strobilus. These primitive features, however, occur only in the terminal portions of the strobilus, which may be regarded as an argument in favor of the "advanced" character of *Taxus*, compared with forms with more abundant centripetal xylem.

Summary

1. The staminate strobili occur in the axils of the leaves. The buds can first be distinguished from other types of buds by the broad apex.

2. The sporophyll primordia first appear as slightly rounded lobes above the general surface and may arise in acropetal succession.

3. The archesporial initials are hypodermal cells and develop according to the eusporangiate method. There are 4–8 of them, distributed around the margin of the primordium.

4. The sporogenous tissue reaches the mother cell stage about October 1, and forms microspores about 2 weeks later. There is no abortion of sporangia such as occurs in *Torreya*, the sporangia occurring in a circle around the stalk of the sporophyll.

5. The sporangium wall is usually 2-layered. The tapetum arises from the peripheral layer of the sporogenous tissue and persists until after megaspore formation.

6. The epidermis of the sporangium remains alive and thin-walled at the base, dehiscence being accomplished by the rupture of these cells at maturity, by the elongation of the stalk of the sporophyll. Owing to the disintegration of the sporangium wall, the epidermis is the functional wall in the later stages.

7. The strobilus matures the latter part of April. Just before maturity there is an enlargement and elongation of the axis, pushing the sporophylls beyond the scales.

8. The strobili of *Taxus canadensis* are somewhat smaller than those of *T. baccata*.

9. The strobilus bundles are collateral endarch, excepting in the terminal portions of the scale bundles and the sporophyll bundles, where they may be mesarch, and in the latter show indications of occasional exarch structure, the terminal portion of these bundles also being concentric.

Huntington, Pa.

LITERATURE CITED

1. Arnoldi, W., Beiträge zur Morphologie der Gymnospermen. III. Embryogenie von *Cephalotaxus Fortunei*. Flora 87:46–63. *pls. 1–3*. 1900.
2. Celakovsky, L., Die Gymnospermen: eine morphologisch-phylogenetische Studie. Abhandl. Königl. Bohm. Gesell. Wiss. VII. 4:1–48. 1890.
3. Chamberlain, C. J., Winter characters of certain sporangia. Bot. Gaz. 25:125–128. *pl. 11*. 1898.
4. Coker, W. C., On the gametophytes and embryo of *Taxodium*. Bot. Gaz. 36:1–27, 114–140. *pls. 1–11*. 1903.

5. COULTER, JOHN M., and LAND, W. J. G., Gametophytes and embryo of *Torreya taxifolia*. BOT. GAZ. **39**:161–178. *pls. 1–3*. 1905.
6. COULTER, JOHN M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. 1910.
7. ———, Morphology of gymnosperms. Revised edition. 1917.
8. DUPLER, A. W., The gametophytes of *Taxus canadensis* Marsh. BOT. GAZ. **64**:115–136. *pls. 11–14*. 1917.
9. GOEBEL, K., Beiträge zur vergleichenden Entwickelungsgeschichte der Sporangien. Bot. Zeit. **39**:697–706, 713–720. *pl. 6*. 1881.
10. ———, Morphologische und biologische Bemerkungen. 13. Über die Pollenentleerung bei einiger Gymnospermen. Flora **91**:237–263. *figs. 19*. 1902.
11. HOFMEISTER, W., Über die Entwicklung des Pollens. Bot. Zeit. **6**:425–434, 649–658, 670–674. *pls. 4–6*. 1848.
12. JEFFREY, E. C., The anatomy of woody plants. 1917.
13. JUSSIEU, A. L. DE, Genera Plantarum. 1789.
14. LAWSON, A. A., The gametophytes, fertilization, and embryo of *Cephalotaxus drupacea*. Ann. Botany **21**:1–23. *pls. 1–4*. 1907.
15. LINDLEY, J., Natural system of botany. 2d ed.
16. LINNAEUS, Genera Plantarum. 6th ed. 1764.
17. NICHOLS, GEORGE E., A morphological study of *Juniperus communis* var. *depressa*. Beih. Bot. Centralbl. **25**:201–241. *pls. 8–17*. *figs. 4*. 1910.
18. PILGER, R., Taxaceae in ENGLER'S Das Pflanzenreich. 1903.
19. RICHARD, L. C., Commentatio botanica de Coniferes et Cycadeis. Posthumous work edited by A. Richard. pp. 20. *pl. 2*. 1826.
20. ROBERTSON, AGNES, Spore formation in *Torreya californica*. New Phytol. **3**:133–148. *pls. 3, 4*. 1904.
21. STARR, ANNA M., The microsporophylls of *Ginkgo*. BOT. GAZ. **49**:51–55. *pl. 7*. 1910.
22. STRASBURGER, E., Die Coniferen und die Gnetaceen. 1872.
23. ———, Die Angiospermen und die Gymnospermen. 1879.
24. ———, Über das Verhalten des Pollens und die Befruchtungsvorgänge bei die Gymnospermen. 1892.
25. ———, Anlage des Embryosackes und Prothalliumbildung bei der Eibe nebst anschliessenden Erörterungen. Festschrift zum siebzigsten Geburtstage von ERNST HAECKEL. pp. 18. *pls. 2*. Jena. 1904.
26. VON MOHL, HUGO, Über die männlichen Blüthen der Coniferen. Verm. Bot. Schriften, pp. 45–61. 1845; published as a dissertation, 1837.
27. WORSDELL, W. C., The morphology of the "flowers" of *Cephalotaxus*. Ann. Botany **15**:637–652. *pl. 35*. 1901.
28. ZUCCARINI, ———, Beiträge zur Morphologie den Coniferen. Abhandl. Acad. München III. p. 794 (from VON MOHL).

EXPLANATION OF PLATES XXIV-XXVI.

All drawings were made with a camera lucida; text figs. 1–10 and 13–22 are drawn to the same scale, with a magnification in reproduction of approximately 36; text figs. 11–12 and all plate figs. are drawn to the same scale, reduced one-half in reproduction, having a magnification of approximately 475.

PLATE XXIV

FIG. 23.—Young sporophyll primordium, showing 2 archesporial initials in hypodermal layer of rounded primordium.

FIG. 24.—Archesporial initial in hypoderm; division of epidermal cell.

FIG. 25.—Archesporial initial in tangential section.

FIG. 26.—Archesporium of 2 cells.

FIG. 27.—Metaphase in division of archesporial initial.

FIG. 28.—Late stage in division of archesporial initial.

FIG. 29.—Primary wall cell (outer cell) and primary sporogenous cell (inner cell), resulting from division of initial.

FIG. 30.—Primary wall cell and division of primary sporogenous cell.

FIG. 31.—Primary wall cell has formed 2 tiers of wall cells, in one of which division is taking place; primary sporogenous cell has divided anticlinally, forming 2 sporogenous cells.

FIG. 32.—Lobe of young sporophyll, showing small mass of sporogenous tissue, 2-layered sporangium wall formed on outer side, and meristematic basal portion of epidermis differentiated from remainder of epidermis.

FIG. 33.—Somewhat older stage than fig. 32.

FIG. 34.—Sporangium wall complete, entirely surrounding sporogenous mass; latter part of July.

FIG. 35.—Older sporangium, showing differentiation of tapetum from sporogenous tissue.

FIG. 36.—Portion of sporophyll, showing epidermis, 2-layered sporangium wall with narrow flat cells, tapetum (1 cell binucleate), and microspores; winter condition.

PLATE XXV

FIG. 37.—Transverse section of portion of lower scale, showing stoma on inner surface and heavily cutinized epidermal walls, especially on outer surface.

FIG. 38.—Transverse section of portion of upper scale, showing vascular bundle and inner epidermis of scale; in vascular bundle note centripetal xylem and 2 transfusion cells, 1 dorsal, 1 lateral to xylem.

FIG. 39.—Transverse section of bundle *a* of fig. 13, showing phloem character of bundle; no xylem present.

FIG. 40.—Bundle *a* at level of fig. 15, showing mesarch character.

FIG. 41.—Bundle *e* of fig. 15; single xylem cell surrounded by phloem.

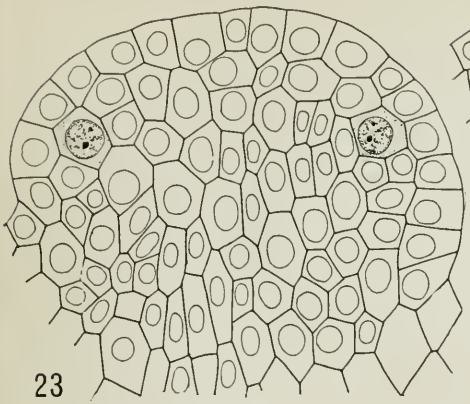
FIG. 42.—Bundle *e* at a lower level; large xylem cell centripetal to smaller ones, indicating possible exarch condition.

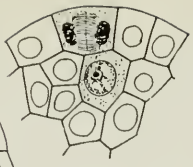
FIG. 43.—Bundle *gb* at level of fig. 17, showing collateral endarch character.

PLATE XXVI

FIGS. 44–47.—Fusion of bundles *f* and *a* (see figs. 15–17): in fig. 44 *f* is concentric, *a*, mesarch collateral; section 60 μ below level of fig. 15; fig. 45, enlarged view of bundle *fa* of fig. 16, 80 μ below fig. 44; fig. 46, 2 bundles near together 20 μ below fig. 45; fig. 47, fusion bundle *fa* 30 μ below fig. 46.

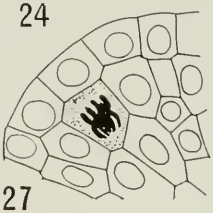
FIG. 48.—Transverse section of bundle *lcm* near level of fig. 22.

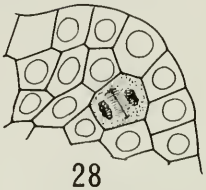

23

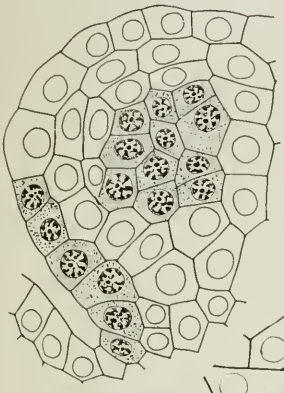

24

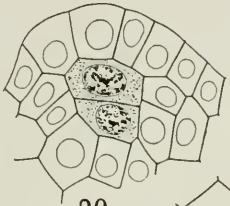
25

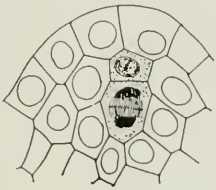
26

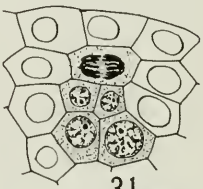

27

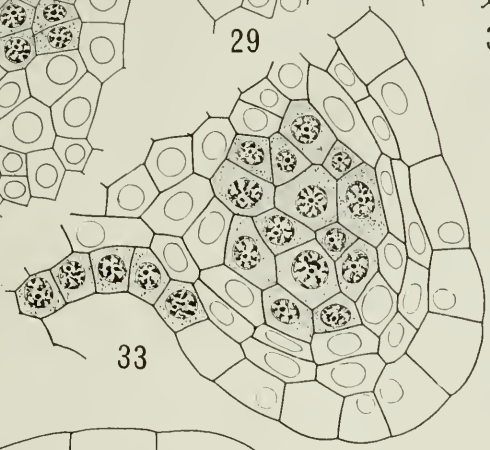

28

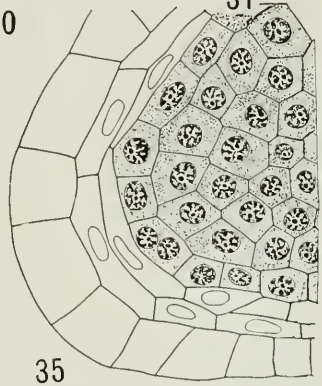

32

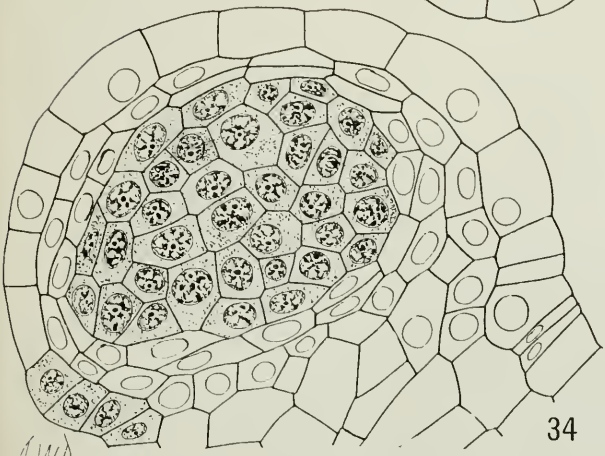

29

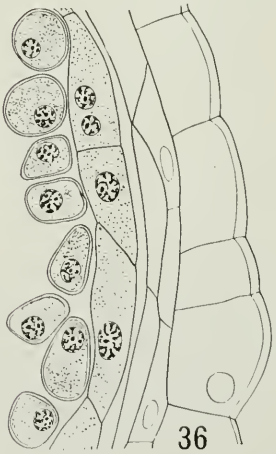

30


31


33

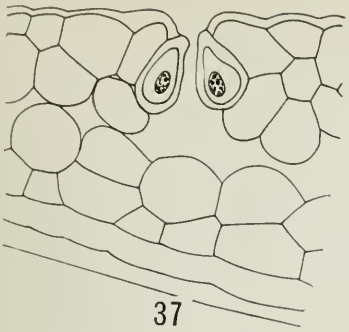

35


34

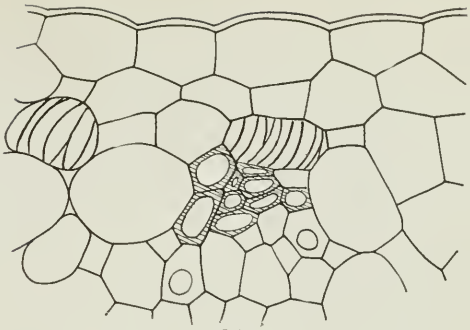

36

AWD

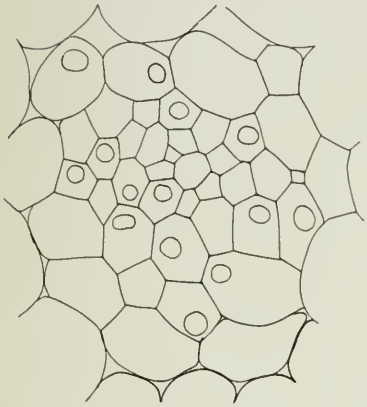
DUPLER on TAXUS



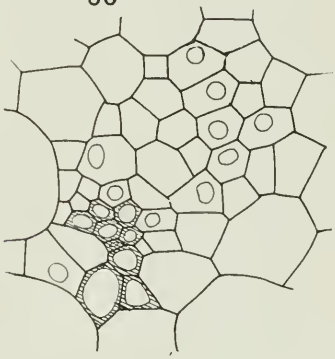
37



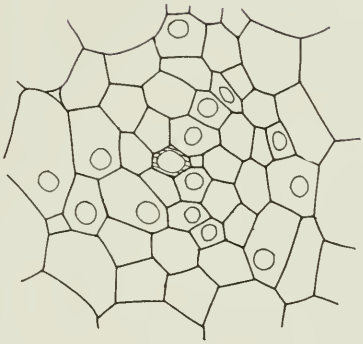
38



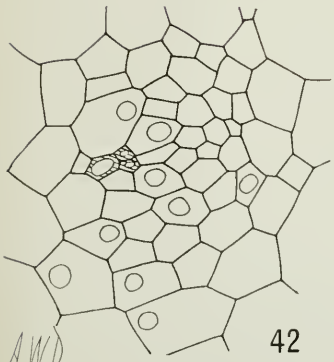
39



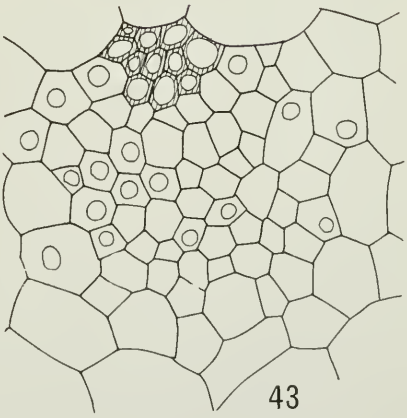
40



41

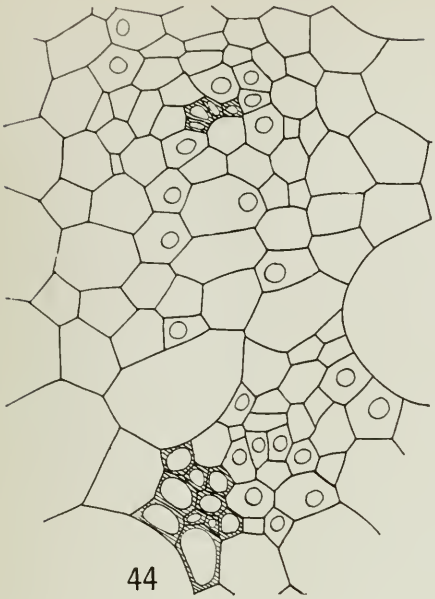


42

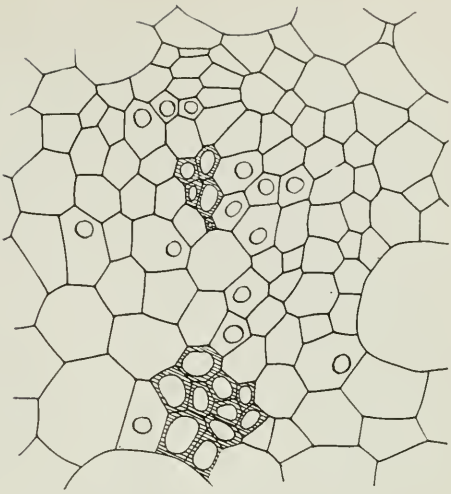


43

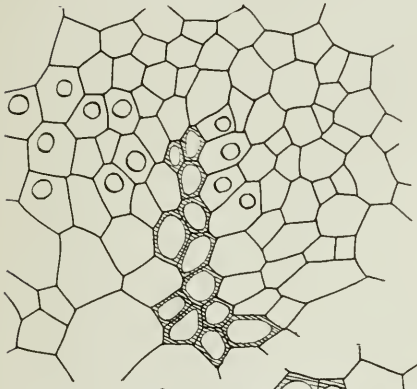
DUPLER on TAXUS



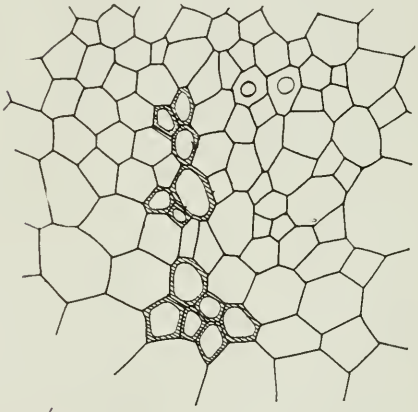
44



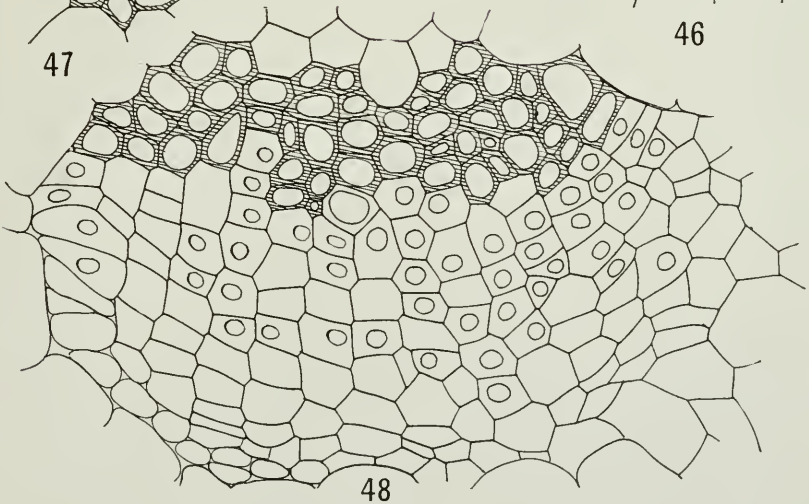
45



47



46



48

A.W.D.

DUPLER on TAXUS

COLLOIDAL PROPERTIES OF BOG WATER

GEORGE B. RIGG AND T. G. THOMPSON

Introduction

This paper is a report of work on the chemical analysis of bog water, the colloidal state of the material in the water, and the effects of this material on the growth of plants. Much of the work is now reported for the first time, but a brief general statement of some of it has been made in a former paper (13). It is also shown how the data here given tend to explain current agricultural practice in bog utilization.

Sphagnum bogs are very numerous in the Puget Sound region and in Alaska. There is scarcely any portion of western Washington in which they are not found, and in some cases there are continuous areas of approximately 300 acres. The fact that these bogs act as a selective habitat and have a peculiar flora of their own, largely xerophytic, makes them objects of great botanical interest, while the fact that the substratum in them, often to a depth of 30 ft. or more, is composed almost entirely of organic matter, and that they have little or no forest covering, makes the utilization of these areas for agricultural purposes a matter of peculiar economic interest.

The acreage of these bogs now utilized for cranberry culture and for gardens, meadows, and pastures, although considerable, is still very small in comparison with their total area in the region. Every bog is a potential crop-producing area of considerable importance, and whatever we can learn with regard to the fundamental factors that govern the growth of plants in bogs in their natural state may function in their transformation into areas in which food production in this region can be increased.

In former papers the senior author has described the flora of some sphagnum bogs of the Puget Sound region (10) and Alaska (11), and has summarized and discussed the various theories (12) that have been suggested to account for the peculiar character of

the flora present in them and the almost complete inhibition from them of plants other than bog xerophytes. Among the various conditions which are factors in this, the toxic properties of the water have been shown to be very important. He has also (13) published evidence tending to show that the toxic properties of this water are due, at least in part, to the presence of matter in a colloidal state. The present paper gives fuller details of the experimental work pointing to this conclusion. It also reports further experimental evidence tending to support this conclusion and furnishes some evidence as to the colloidal state in which the toxic substances are present.

Experimental work

The samples of bog water used were collected in most cases by digging a hole, usually not more than 30 inches deep, in the substratum, and dipping from this the water that accumulated within a few minutes. When the bogs were too dry to admit of securing water in this way it was squeezed from handfuls of the decaying material. In all cases it was collected in glass containers and taken to the laboratory, where it was strained through washed cheesecloth and then filtered twice through filter paper.

Samples collected from several bogs and filtered in this way were treated with various electrolytes [NaCl, $MgSO_4$, $(NH_2)SO_4$, and Na_2HPO_4] to determine whether there was any material in the water that could be precipitated by this means.

To a 250 cc. sample from North Mud Lake bog a like volume of saturated solution of $(NH_2)SO_4$ was added. After shaking thoroughly this was allowed to stand. At the end of 2 days no precipitate had appeared, but at the end of 5 days a precipitate could readily be seen.

When 90 cc. of water from Fauntleroy bog was saturated with $(NH_4)_2SO_4$ by adding the salt gradually and shaking no precipitate appeared at once. When this had stood over night, however, there was a considerable quantity of precipitate, consisting of brown, somewhat flaky particles, some at the surface, some at the bottom, and some remaining suspended in the liquid. This precipitation by complete saturation with $(NH_4)_2SO_4$ was repeated

with two samples from the North Mud Lake bog and one sample from each of the following bogs: Maltby bog, Henry bog, and the bogs at Cordova, Alaska, and Sand Point, Alaska. The results in all cases were the same. The work was then repeated by saturating samples from these same bogs with NaCl, $MgSO_4$, and Na_2HPO_4. The results in all cases were the same qualitatively as when $(NH_4)_2SO_4$ was used, although the quantity of precipitate varied somewhat. Later precipitation of samples from these bogs and other bogs of the Puget Sound region was tried repeatedly, and in all cases the results were as described.

The precipitate from 7 samples treated with $(NH_4)_2SO_4$ was filtered off on filter paper. Each filtrate was then placed in a dialyzing tube of parchment paper and dialyzed in running water until the contents of the tubes showed no precipitate with barium chloride. The toxicity of these filtrates was then tested by growing cuttings of *Tradescantia* in them in the same way that the toxicity of bog water had been tested earlier (10). Controls of *Tradescantia* cuttings in untreated bog water and in Cedar River water were run. The root hairs developed well in the filtrate and in the Cedar River water, while their development was very poor in the bog water. No difference could be seen between those grown in the filtrate and those in the Cedar River water. Samples of bog water were also dialyzed and cuttings of *Tradescantia* were grown in them. They were still just as toxic as untreated samples.

On January 25, 1918, 6 samples of bog water that had been collected from Henry bog on November 5, 1916, and filtered through filter paper on November 7 and again on November 9, 1916, were all found to contain considerable quantities of a brown precipitate in irregular, somewhat flaky, masses. A sample collected from Maltby bog on November 30, 1915, and filtered December 4, 1915, showed the same results, as did also one collected at Sand Point, Alaska in July 1913, and filtered October 27, 1915. A gradual aggregation of the colloidal material occurred with the lapse of time in every case.

In order to test the volatility of the toxic substances, 500 cc. of the filtered bog water was distilled on a water bath until the residue was only 80 cc. The distillate was colorless, while the

concentrate was much darker than bog water. The material in the concentrate all remained in solution. No precipitate appeared and no incrustation was left in the beaker when the concentrate was poured out. The toxicity of the distillate and of the concentrate was tested by growing *Tradescantia* cuttings in samples of them. The concentrate proved to be more toxic than the untreated bog water, while root hairs developed as well in the distillate as they did in Cedar River water. Samples of the concentrate and of the distillate were saturated with solid $(NH_4)_2SO_4$ and allowed to stand overnight. The distillate gave no precipitate in any case, while the concentrate gave a much heavier precipitate than did the untreated bog water.

Since all samples of bog water tested had been found acid to litmus and to phenolphthalein, tests of both the concentrate and the distillate were made by titrating with N/20NaOH, using the same indicators. The acidity of the concentrate was in every case found to be greater than that of untreated bog water. The distillate was slightly acid, but much less so than the untreated water. The precaution of removing the CO_2 by boiling before titrating was taken in each case. Since both the concentrate and the untreated bog water are colored solutions, the use of indicators with them is unsatisfactory, and more exact means would have to be used in order to get quantitative data. Table I summarizes most of these results, together with some presented later in the paper. The letters A, B, and C indicate relative acidity, A being greater than B, while C is much less than B.

TABLE I

Constituents	Precipitate with electr.	Toxicity	Acidity
Bog water	+	+	B
Distillate	−	−	C
Concentrate.....	+	+	A
Residue	+	+	B

Work was next undertaken to secure data on the chemical constitution of the materials in solution in the bog waters that had been experimented on and to get some indication as to what

the colloidal state of the toxic material is. Six samples of water were collected for analysis. The usual precautions recommended for the sampling of waters were rigidly followed. The localities were as follows: (1) the swamp adjoining Henry bog, (2) a point approximately in the center of Henry bog some 800 or 900 ft. from the edge of the swamp, (3) a point in West Mud Lake bog about 600 ft. from its edge, (4) another point in the same bog about 500 ft. from where sample 3 was obtained, (5) a very narrow strip of swamp land lying between the points where samples 3 and 4 were obtained, (6) a few inches under the surface of Lake Washington, a short distance from shore, where the water was about 30 ft. deep. These samples are referred to in table II by these numbers.

TABLE II

ANALYSIS OF WATERS FROM SWAMPS, BOGS, AND LAKES

Constituents	1	2	3	4	5	6
Total organic nitrogen	2.92	3.09	2.17	2.34	2.31	0.834
Nitrogen as albuminoid ammonia	0.855	0.850	1.20	0.526	0.500	0.20
Nitrogen as free ammonia	0.52	0.013	0.472	0.420	0.175	0.108
Nitrogen as nitrites	0.005	0.003	0.010	0.010	0.012	0.0002
Nitrogen as nitrates	0.05	0.05	0.040	0.040	0.030	0.06
Chlorine	0.96	1.44	0.708	0.743	0.702	2.70
Oxygen required	42.50	41.50	14.50	14.30	16.40	7.55
Total solids	109.00	101.20	92.00	93.00	107.00	65.00
Loss on ignition	83.50	85.70	62.00	65.00	64.00	20.00

Sample 1 possessed a strong earthy odor and a light straw color. It was collected so close to the edge of the bog as to be considerably influenced by the character of the water of the bog. Sample 2 had a very slight odor, and its color was much deeper than that of sample 1. Samples 3, 4, and 5 all possessed a slight earthy odor and had a light straw color. Sample 6 was perfectly clear and had no odor. Samples 1 and 2 were collected February 20, 1917; samples 3, 4, and 5 were collected March 13, 1917; sample 6 was taken during the spring of 1917. The various constituents of the waters examined were determined according to the methods given in "Standard methods for the examination of water and sewage," used by the American Health Association.

Determinations of the amount of solid matter and of the required oxygen on a few samples of water from other Puget Sound bogs gave results lying within the limits of the values reported for samples 3, 4, and 5. While only one analysis of Lake Washington water is here shown, the junior author has made frequent analyses of lake water in this region and has found them consistent with the analysis here given for Lake Washington water.

In table III the values in column 1 are an average of those in columns 2, 3, and 4 of table II. The values in column 2 are the same as those in column 6 of table II.

TABLE III

COMPARISON OF BOG WATER AND LAKE WATER

Constituents	1 (bog)	2 (lake)
Total organic nitrogen	2.53	0.0834
Nitrogen as albuminoid ammonia	0.855	0.200
Nitrogen as free ammonia	0.301	0.108
Nitrogen as nitrites	0.008	0.0002
Nitrogen as nitrates	0.043	0.06
Chlorine	0.963	2.70
Oxygen required	23.400	7.55
Total solids	95.400	65.00
Loss on ignition	70.900	20.00

FOULK (7) has made two analyses of Ohio bog water. A period of two years elapsed between the time of taking the two samples, which were from the same bog. The two analyses differ considerably, but both agree with the ones here reported in showing a large oxygen requirement and a large loss on ignition.

Several samples of Puget Sound bog water were evaporated to dryness in porcelain dishes on a water bath. A dark brown powdery residue was obtained. Other samples were evaporated to dryness in a porcelain dish heated over a Bunsen burner and protected only by a thin piece of asbestos and wire gauze. The residue so obtained was of the same appearance as that from the water bath evaporation. Some of the residue from the water bath evaporation was heated to redness in a porcelain dish. It gave no protein odor as it burned. The biuret test for protein

was applied to other portions of this residue. The results were negative in every case. The solubility of this residue was then tried. It dissolved readily in cold Cedar River water. Solution was complete in a volume of Cedar River water equal to the volume of bog water from which the solid matter was obtained. The toxicity of this solution of solids in Cedar River water was tested by growing cuttings of *Tradescantia* in it, with other cuttings in bog water and Cedar River water as controls. The bog water and the solution of bog solids greatly reduced root hair development on the cuttings, while root hairs developed abundantly on cuttings in Cedar River water. The water solution of this residue was found to reduce Fehling's solution slightly.

The brownish residue was found to be insoluble in alcohol and gasoline. In all cases but one it was also found entirely insoluble in ether. In this one case enough went into solution to impart a brownish color to the porcelain dish in which the ether was allowed to evaporate. When bog water was shaken with an equal volume of ether in a separatory funnel nothing was extracted from it.

An attempt was made to throw out the solid matter in bog water by centrifuging it. Samples were centrifuged for 20 minutes at 1800 revolutions, but no solid matter at all was thrown out.

Discussion

The experimental data furnish evidence as to the substances present in the water of sphagnum bogs, and also certain indications in regard to the colloidal state of the soil solution in them and the relation of this colloidal material to the toxicity of the water. They have also a considerable bearing on agricultural utilization of these areas.

The large amount of organic matter in bog water as compared with lake water is clearly shown by the large amount of solid matter and the loss sustained when this solid matter is heated to full redness. The solid matter in bog water is 146 per cent of that in lake water. The solid matter from bog water lost 74 per cent of its weight on ignition, while that from lake water lost only 30 per cent. That this organic matter is still in a very limited state of oxidation is indicated by the large amount of oxygen that

is required. More than three times as much oxygen was required by the bog water as by lake water.

The large amount of solid organic matter in bog water is evidently the result to a large extent of the breaking down of plant tissues in the absence of an adequate supply of oxygen. The interstices of the decaying mass of material are full of water at all times at a depth of 2 ft. or more, and even in the first 2 ft. except occasionally for a brief time in midsummer. Since organisms are abundant in bogs, it seems evident that the nature of the products must be conditioned by at least 3 factors: (1) the original composition of the decaying materials, (2) the organisms present, (3) the environmental conditions under which they act. DACHNOWSKI (**5, 6**) has emphasized the reducing power of bog soils, and has found that aeration lowers the toxicity of the water to agricultural plants. KLEIN (**9**) had made suggestions along this line as early as 1880.

A very large amount of the nitrogen present in bog water is in the organic form. The total organic nitrogen content of the bog water here reported is over three times as much as that of the lake water reported. In the bog water the total organic nitrogen is 50 times the combined nitrite and nitrate content, while in the lake water it is only 14 times.

The work of JODIDI (**8**) and of ROBINSON (**16**) show large amounts of nitrogenous matter in bog soils, and indicate that it is either already in the form of amino acids and acid amides, or is capable of being converted readily into these compounds.

The amount of nitrogen as nitrates is slightly larger in the lake water than in the bog water. Even if this difference were large it could not be accepted as necessarily indicating a difference between the nutritive value of the two waters for plants, since it is well known that some organic compounds are beneficial to plant growth (**2, 4, 17, 19**). A number of these beneficial organic compounds are nitrogenous. BOTTOMLEY'S (**2**) work indicates the abundance of such a compound in sphagnum peat that has been acted upon by aerobic soil organisms.

The amount of nitrogen as free and albuminoid ammonia varies considerably in the different analyses, and the existence of

nitrogen in these conditions is apparently influenced to a large extent by immediate local conditions.

KLEIN (9) was the first one to suggest that the toxicity of bog water is due to the presence of chemical combinations harmful to plant life. The water from a black bog overflowed into the meadows of a certain portion of East Prussia, causing considerable damage. He analyzed this water and found 312.8 parts per million of organic matter and 175.9 parts per million of inorganic matter. Among the reasons which he points out as to why this water is injurious to crop production these two are of special interest: (1) it acts as a reducing agent, (2) it produces chemical compounds that are harmful to plant life. The bog he studied differed from Puget Sound bogs in having considerable mineral content. Our bogs and his have in common the avidity of the organic matter for oxygen. His suggestion of toxicity is the earliest one seen by the authors. The review of his work says that when exposed to air the moist peat soon took up oxygen, with the result that there was formed on the surface of the soil a hard crust which was "impervious to the oxygen of the air, and the humus, withdrawing oxygen from the iron compounds, formed salts destructive to vegetable life." It does not seem positive from this wording whether the "salts destructive to vegetable life" came from the iron compounds or from the humus.

Previously published results (15) indicating that the osmotic pressure of bog water is very low had suggested that the material in solution in it is probably in a colloidal state. The data given seem to confirm this view, while the experiments with *Tradescantia* cuttings indicate that the matter in the colloidal state is a large factor in the toxicity of the water.

BAUMAN and GULLY (1) have shown that the acidity of bog water is due to the colloidal material of the cell walls of the hyaline cells of sphagnum. This would seem to suggest that both the acidity and the colloidal properties of bog water are due to the breaking down of sphagnum. The senior author has suggested in an earlier paper (10) that since the essential conditions for the formation of a bog are the continued growth of sphagnum and the

lack of drainage, the place to look for the origin of the toxicity is in the decay of sphagnum under anaerobic conditions. It seems probable (but is not proved) that the acidity, the colloidality, and toxicity all have their origin here.

It seems clear that the substances in bog water that are precipitated by electrolytes and on long standing without electrolytes, and will not dialyze through parchment paper, and, although present in considerable quantities, do not appreciably lower the freezing point of the water, are in a colloidal state. Since bog water and preparations from it (for example, the concentrate and the solution of the residue from evaporation) which contain these substances are toxic to *Tradescantia* cuttings, while preparations that do not contain them (the distillate) are not toxic, it appears that the toxicity is associated with the matter that is in a colloidal state. Concerning the nature of the colloid we have the following indications.

1. The colloid is thermo-stable. This is indicated by the fact that the distillate is non-toxic, while the concentrate is more toxic than untreated bog water.

2. The colloid is reversible, because when obtained as solid matter it is quickly redissolved on the addition of a volume of cold water equal to the volume of bog water from which the solid was originally obtained.

3. The solid material of bog water when obtained in the dry form is a granular powder. Since the distillate from which this was obtained as a residue is not colloidal, and since a solution having colloidal properties can be obtained by redissolving this solid matter, it seems that it is the material that is in the colloidal state.

4. Bog water has elsewhere (15) been shown to have a slightly lower surface tension than that of pure water. To what extent this may be due to the presence of the colloid has not been determined.

5. No means has been found of testing the swelling of this colloid, since it goes into solution so readily.

6. No evidence has been found that the colloids increase the viscosity of the water.

One means used in the Puget Sound region for bringing these bogs into cultivation is to drain them, scalp off and remove the living vegetation at the surface to a depth of 8 or 10 inches, and then stir up and aerate the partially decayed matter underneath. It seems probable that the success of this plan finds its explanation largely in the removal of much of the toxic material in draining off the water, and the oxidation of the remainder to non-toxic compounds when the soil is exposed to the air.

The work of BOTTOMLEY indicates that a substance or substances beneficial to the growth of higher plants and also increasing the rate of nitrogen fixation by soil organisms results from the action of aerobic soil organisms on sphagnum peat. To what extent the beneficial effects of the aeration of bog soils may be due to oxidation independent of organisms, and to what extent it may be due to the action of organisms under the changed conditions, have not been determined. It seems likely that crop plants in bogs that have been brought into cultivation are influenced beneficially by these compounds. To what extent this beneficial effect may result from actual use of these compounds as definite constituents of plant foods, or to what extent it may be due to their general catalytic effects or their part in certain definite types of metabolism, is not known. SCHROEDER (18) found that the direct application of peat alone to sandy soils gave increased yield of crops, although still better results were obtained when lime and stable drainings were used with the peat. Another practice for bringing these bogs into cultivation is that of destroying the surface vegetation by fire during the dry season. Usually the fire does not penetrate far into the sphagnum substratum because of the moisture beneath.

This practice results, of course, in the practically complete oxidation of the material in the surface layer of the bog. The preparation of the burned-over bog for the planting of crops and the subsequent cultivation of these crops secure aeration and consequent oxidation of the unburned material and also render the soil lighter by mixing the ashes with it. The acidity of the soil is neutralized to a considerable extent by the basic properties of the ash when it goes into solution.

In the main, however, the success that has been attained in the cultivation of bog lands in the Puget Sound region has been attained by the use of acid-tolerant crops (3) such as cranberries, strawberries, celery, onions, lettuce, cabbage, and carrots, rather than by correcting the acidity in order to grow crops that are not acid tolerant.

Summary

1. Bog water gives a precipitate on standing a few hours after saturation with electrolytes.

2. It also gives a precipitate on standing a year or more without electrolytes.

3. The filtrate from the precipitation with $(NH_4)_2SO_4$, when dialyzed until free from sulphates, is not toxic to the root hairs of *Tradescantia* cuttings.

4. Bog water, when dialyzed for the same length of time as this filtrate, is toxic to these root hairs.

5. The distillate from bog water gives no precipitate with electrolytes, is much less acid than bog water, and is not toxic to these root hairs.

6. The concentrate obtained when bog water is distilled to approximately one-sixth of its original volume gives a heavier precipitate with electrolytes than does bog water. It is also more acid and more toxic to these root hairs.

7. The residue from complete evaporation of bog water is a brownish powder which is soluble in cold water, insoluble in alcohol and gasoline, and practically insoluble in ether.

8. This water solution of the residue is toxic to the root hairs of *Tradescantia*.

9. No solid matter was thrown out of bog water by centrifuging.

10. Chemical analyses of Puget Sound bog waters give results similar to those reported for other American bog waters.

11. The toxicity of bog water to *Tradescantia* cuttings seems to be connected with the matter in it that is in a colloidal state.

12. The oxidation of this toxic matter to non-toxic matter seems to be a basis of agricultural practice in bringing bog lands into cultivation.

UNIVERSITY OF WASHINGTON
SEATTLE, WASH.

LITERATURE CITED

1. BAUMAN, A., and GULLY, E., Über die freien Humus Saeuren des Hochmoors. Mitt. K. Bayr. Moorkulturanst. 4:31–56. 1910.
2. BOTTOMLEY, W. B., The significance of certain food substances for plant growth. Ann. Botany 28:531–540. 1914; also Proc. Roy. Soc. B. 88:237–247. 1914.
3. COVILLE, F. V., The agricultural utilization of acid lands by means of acid tolerant crops. Bull. 6. U.S. Dept. Agric. 1913.
4. CURTIS, O. F., Stimulation of root growth in cuttings by treatment with chemical compounds. Mem. 14. Cornell Univ. Agric. Exp. Sta. 1918.
5. DACHNOWSKI, A., The toxic property of bog water and bog soil. BOT. GAZ. 46:130–143. 1908.
6. ———, Bull. 16. Geol. Survey Ohio. 1912.
7. FOULK, C. W., Bull. 16. Geol. Survey Ohio. 1912.
8. JODIDI, S. L., Organic nitrogenous compounds in peat soils. Mich. Agric. Coll. Exp. Sta. Tech. Bull. 4. 1909.
9. KLEIN, Bied. Centr. 1880, pp. 168–171; rev. Jour. Chem. Soc. London 38:738. 1880.
10. RIGG, G. B., The effect of some Puget Sound bog waters on the root hairs of *Tradescantia*. BOT. GAZ. 55:314–326. 1913.
11. ———, Notes on the flora of some Alaskan sphagnum bogs. Plant World 17:167–182. 1914.
12. ———, A summary of bog theories. Plant World 19:310–325. 1916.
13. ———, The toxicity of bog water. Science II. 48:602. 1916.
14. ———, Forest succession and rate of growth in sphagnum bogs. Jour. Forestry 15:726–739. 1917; see also BOT. GAZ. 65:359–362. 1918.
15. RIGG, G. B., TRUMBULL, H. L., and LINCOLN, MATTIS, Physical properties of some toxic solutions. BOT. GAZ. 61:408–416. 1916.
16. ROBINSON, C. S., Organic nitrogenous compounds in peat soils. Mich. Agric. Coll. Exp. Sta. Tech. Bull. 7. 1911.
17. SCHREINER, O., The organic constituents of soils. Science II. 36:577–587. 1912.
18. SCHROEDER, J., Bied. Centr. 1879, pp. 634–635; rev. Jour. Chem. Soc. London 38:506. 1880.
19. SKINNER, J. J., and BEATTIE, J. H., Effect of asparagin on absorption and growth in wheat. Bull. Torr. Bot. Club 39:429–437. 1912.

VEGETATION OF UNDRAINED DEPRESSIONS ON THE SACRAMENTO PLAINS

FRANCIS RAMALEY

(WITH ONE FIGURE)

The observations here recorded were made in 1917 from March to May inclusive, chiefly in the neighborhood of Sacramento, California. Study, however, was extended in all directions for distances of 20 miles or more, and even to Chico, 90 miles north, and to Stockton, 40 miles south.

Most of the area is exceedingly flat and low. The city of Sacramento itself is at an altitude of 30 ft. above sea-level. Except to the east, in the Sierra foothills, there is scarcely a rise of 100 ft. within 25 miles. Soils are largely sand, sandy loam, sandy clay, and clay loam. Any fine grained soil which retains water is popularly known as "adobe," the term not being confined to clay.

The numerous shallow depressions[1] of the Sacramento plains[2] have arisen from the leaching out of limestone masses. They are of various extent, some only a few square meters in area and others 100 m. or more across, with a depth of a few centimeters or decimeters. The soil is very fine grained and holds water for an astonishingly long time after rains, often for one or two weeks. During the rainy period of winter these low places may be constantly full of water. Even with the lessened rainfall of early spring there is likely to be standing water during much of the month of March. Besides these vernal pools there are many shallow "draws," or ravines, having a vegetation cover which bears a resemblance to that of the undrained depressions. As would be expected, however, they have many more species of plants because

[1] These depressions could hardly escape the notice of botanists. Mention of their flora is made in the preface (p. 5) of JEPSON'S "Flora of Middle Western California," ed. 2. 1911.

[2] The term "Sacramento plains" is well established in botanical and popular writings, but these are not "plains" in the usual botanical sense. The vegetation is rather that of a vernal meadow or vernal prairie. During the growing period of March, April, and May the soil moisture is sufficient for the support of true mesophytic species; witness the abundance of European weeds.

of the more favorable edaphic conditions. The present paper will describe only the undrained depressions.

Early in March a spring outburst of flowering annuals occurs on the Sacramento plains. A conspicuous flora develops, which continues, with various changes, during two or three months. This vernal meadow-grassland shows great masses of color, due to the flowers of *Calandrinia*, *Eschscholtzia*, *Platystemon*, *Trifolium*, *Orthocarpus*, *Nemophila*, *Erodium*, *Lupinus*, *Gilia*, *Brodiaea*, *Layia*, *Lotus*, *Collinsia*, etc. Toward the latter part of May these annuals and the various grasses have ripened their seed, and the landscape has become brown.

The low places in the plains show no fresh vegetation for two or three weeks after the spring outburst takes place in the ordinary grassland, for the ground is still wet and cold. At 2 dm. the soil temperature is 12–15° C., instead of 18–21° as in the vernal meadow.

The earliest plant of the depressions is the "meadow-foam," *Floerkea Douglasii* (Limnanthaceae). This is a succulent herb about 1 dm. tall, with a profusion of white or roseate flowers. It shows first as a fringe at the margin of low areas, extending around on a contour line and resembling foam on a windy shore (fig. 1). Later, as the soil becomes drier and warmer, the meadow-foam works inward, meeting the vegetation that now appears in the central part of the depression. By May 1, at Sacramento, the meadow-foam has ceased blooming and has well developed fruits. Not all depressions have the fringe of meadow-foam here described, but very many do.

Two rather definite areas may be distinguished in the depressions. There is a central portion which remains wet for a longer period, and a marginal area around it which merges into the surrounding ordinary grassland. The central and marginal areas have their own characteristic plants. A condensed statement later in this article indicates seasonal differences in the appearance of the two areas.

Central area

The central area is characterized as a rule by a growth of *Allocarya californica*, a low, white-flowered borage, or sometimes by other species of the genus. Often the *Allocarya* forms a close

stand, with no other plants present.[3] The *Allocarya*, however, is sometimes scattered, and the chief mass of vegetation is "gold-fields" (*Baeria* or *Lasthenia*). Because of the almost constant occurrence of *Allocarya*, these plant communities might well be called "*Allocarya* depressions." The *Allocarya* continues in bloom until late in the growing season, even after the general spring aspect has passed. Many of the plants of this late period

FIG. 1.—A depression in early spring showing a wide circum-area of meadow-foam (*Floerkea Douglasii*); outskirts of Sacramento.

are markedly depauperate in vegetative parts, and also as to size of flowers; but even during the early part of the season depauperate individuals are scattered among the more robust plants.[4]

Gold-fields (*Baeria* and *Lasthenia*) sometimes replace almost completely the *Allocarya* of the central area. At a distance they

[3] The writer is familiar with such growths of *Allocarya* at Tolland, Colorado, in the Rocky Mountains along lake and stream margins in fine grained soil. The Colorado species in these places is *Allocarya scopulorum*.

[4] Many Californian species have depauperate or otherwise atypical individuals mixed with plants of typical form, or in some cases occurring in special habitats. It is often difficult to determine in a given case whether the differences are genetic or merely due to crowding or other environmental factors.

may be mistaken for some low-growing *Ranunculus*, because of the profusion of yellow flowers. These plants are found where there is some loose loamy soil above the close grained adobe, or where there is some drainage, as in a shallow draw. If the soil at the center of the depression is fine adobe, then the *Baeria*, if present, will form a ring outside the centrally placed *Allocarya*. Or, if water stands an unusually long time in the center, there may be a growth of *Damasonium californicum* (Alismaceae), bounded by a ring of *Allocarya*, and this in turn by a circum-area of *Baeria*.

Within the inner area of the depressions *Downingia*, a small Lobeliaceous plant, becomes conspicuous in late spring because of its great abundance. Even at a distance it may be recognized by its pale blue tint, for it sometimes fills nearly the entire central part after the flowers of *Allocarya* have disappeared. In some cases the *Downingia* develops as a wide circum-area, not quite reaching the center of the depression. Often associated with *Downingia*, and sometimes replacing it, is *Gilia leucocephala*, which when in large masses gives a whitish tinge to the vegetation complex.

"Coyote thistle" (*Eryngium Vaseyi*) is a prickly umbellifer with inconspicuous flowers which occurs in the central part of most of the depressions. Occasionally it forms a rather dense growth, but is more often rather loosely scattered. Hence, although it is the largest of the plants found at any time in the depressions, it has little influence upon the general appearance of the vegetation until late in the season, when it has grown tall and leafy, and when the showy annuals have completed their growth and dried up. The coyote thistle plants have a glaucous appearance that dominates the depressions, replacing the pale blue of *Downingia*, which followed the white of *Allocarya* or the yellow of gold-fields.

Among the less abundant plants of the inner area is the "freckled monkey" (*Mimulus angustatus* and *M. tricolor*). The plants are small, even minute, and top-heavy with large purple flowers spotted with yellow. When not prominent because of numbers the freckled monkey can nearly always be found rather late in April if search is made for it on the surface of the dried mud among the coyote thistles. Another small plant often abundant, and

practically always represented by at least a few specimens, is *Psilocarphus brevissimus*, a whitish-headed composite scarcely 1 cm. tall.

Marginal circum-area

The marginal zone is characterized in late spring by *Deschampsia danthonioides*. Sometimes this grass forms an almost pure stand after the disappearance of the meadow-foam, which, it will be remembered, marked the marginal zone in the first part of the season. As would be expected, the marginal zone is subject to invasion from within and from without, but it remains, as a rule, a very definite and distinct entity.

The inner boundary of the marginal circum-area is sometimes made by a thin line of *Achyrachaena mollis*, an inconspicuous composite that becomes noticeable in fruit by the spreading out of its silvery pappus. This same narrow line may be marked a little later in the season by a scattered ring of a kind of tarweed, *Hemizonia Fitchii*. Neither the *Achyrachaena* nor the tarweed, however, are essential elements in the circum-area; often they are entirely absent.

The ordinary drained areas of the Sacramento plains have a large proportion of introduced plants, especially grasses, bur clover, and species of *Erodium*. This is true of abandoned fields and of areas never under cultivation. The depressions, on the contrary, have an almost strictly native vegetation. If a patch of cultivated ground is left untouched, the depressions soon return to their original condition, unless the field has been thoroughly manured and cultivated. This recrudescence of the original vegetation, of course, is readily possible where most of the species are annuals. A definite case of return of native flora was noted in North Sacramento. An abandoned field had grown up to European grasses and weeds, but a low place in it, which plainly showed the marks of previous cultivation, was now like any of the primitive untouched adobe depressions. On May 15, 1917, the vegetation of the central area, about 10 m. across, was chiefly *Allocarya* and *Eryngium*, with a small amount of *Mimulus*, *Downingia*, *Gilia leucocephala*, and *Deschampsia danthonioides*. Outside this central part was a wide circum-area dominated at this time by *Deschampsia*,

with dried plants of meadow-foam, showing that the early spring aspect also had been of the usual type. Subordinate elements of the marginal ring were chiefly introduced weeds, *Silene*, *Lepidium*, *Hypochaeris*, *Rumex*.

The changing appearance of the depressions following the advance of the season may be shown best by a tabular statement.

PREVERNAL ASPECT

CENTRAL AREA.—Scattered plants of *Eryngium* left over from the previous season, but most of the ground bare.

MARGINAL ZONE.—*Floerkea* forming a more or less dense ring extending out to the ordinary grassland. The ring is often invaded toward the outside by species of the adjacent area, such as various native clovers, *Orthocarpus*, and European weeds.

MID-VERNAL ASPECT

CENTRAL AREA.—*Eryngium* actively growing but not yet of full size. *Allocarya* develops and blooms at the true beginning of spring; often forms a close community. *Baeria* and *Lasthenia*, if present at all, are likely to appear early. *Mimulus* sparingly present. Young plants of *Alopecurus* becoming abundant.

MARGINAL ZONE.—*Deschampsia* now showing definitely as a close growth of young plants. Native clovers in flower and fruit. Young plants of *Achyrachaena*. Introduced weeds.

LATE VERNAL ASPECT

CENTRAL AREA.—*Eryngium* now taller and more conspicuous than before. *Allocarya* continued from the previous aspect, but the plants now in bloom are depauperate. *Baeria* and *Lasthenia* in fruit or now drying up, with a few belated ones still blooming. *Alopecurus*, *Mimulus*, *Gilia leucocephala*, *Psilocarphus*, and *Downingia* in flower and fruit. In many places the last named very abundant and giving character to the entire depression.

MARGINAL ZONE.—*Deschampsia* in flower and fruit. *Downingia* scattered through the inner part of the marginal zone. Native clovers and *Orthocarpus* in fruit. *Achyrachaena mollis* in fruit.

At the outer boundary of the zone various introduced weeds now in flower and fruit.

Systematic list

The species of the depressions are recorded in the following list. Reference letters have the following significance: *C*, characteristic; *F*, frequent; *O*, occasional.[5]

Alismaceae
Damasonium californicum (F. and M.) Greene, *O*

Poaceae
Alopecurus californicus Vasey, *F*
Alopecurus geniculatus Linn., *F*
Deschampsia danthonioides Munro, *C*
Phalaris Lemmoni Vasey, *O*

Cyperaceae
Eleocharis acicularis R. Br., *O*

Juncaceae
Juncus tenuis Willd., *O*

Limnanthaceae
Floerkea Douglasii Baill., *C*

Lythraceae
Lythrum hyssopifolium Linn., *O*

Onagraceae
Boisduvallia stricta (Gray) Greene, *O*

Apiaceae
Eryngium Vaseyi C. and R., *C*

Polemoniaceae
Gilia intertexta Steud., *O*
Gilia leucocephala Benth., *C*

Boraginaceae
Allocarya californica (F. and M.) Greene, *C*
Allocarya stipata Greene, *O*
Allocarya trachycarpa (Gray) Greene, *F*

Lamiaceae
Pogogyne ziziphoroides Benth., *O*

Scrophulariaceae
Mimulus angustatus Gray, *F*
Mimulus tricolor Hartw., *C*

[5] The writer is under obligation to Professor HARVEY M. HALL for the privileges of the University of California Herbarium, and to both Professor HALL and Miss WALKER in naming for him a considerable collection of Californian plants.

Lobeliaceae
Downingia pulchella (Lindl.) Torr., *C*

Carduaceae
Achyrachaena mollis Shauer, *F*
Baeria chrysostoma gracilis (DC.) Hall, *C*
Baeria Fremontii Gray, *C*
Baeria platycarpha Gray, *F*
Blennosperma californicum (DC.) T. and G., *O*
Hemizonia Fitchii Gray, *O*
Lasthenia glabrata DC., *O*
Psilocarphus brevissimus Nutt., *C*

Cichoriaceae
Hypochaeris glabra Linn. (introduced), *F*

Summary

The numerous depressions of the Sacramento plains have a very fine grained soil, where water stands during the period of winter rain and even well into early spring. The vegetation is very different from that of the usual grassland of the region, being composed of very few species, with practially no introduced weeds. The depressions usually show a central area and a marginal zone, the former characterized by a dense growth of *Allocarya* or *Baeria*, and the latter by *Floerkea Douglasii* and *Deschampsia danthonioides*. Subordinate species of both areas are noted and the seasonal changes indicated. A systematic list is given of 29 species, 10 of which are marked as characteristic, 8 as frequent, and 11 as merely occasional.

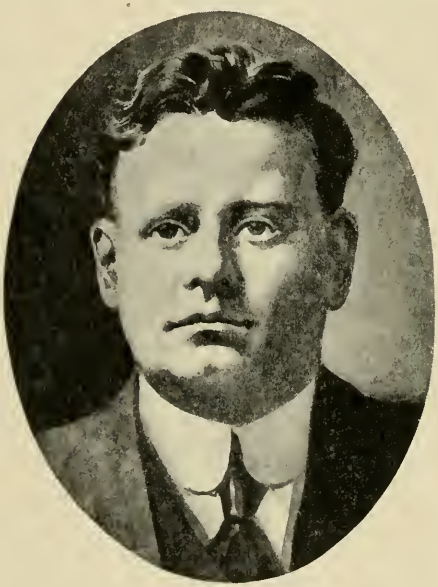
University of Colorado
Boulder, Colo.

BRIEFER ARTICLES

AARON AARONSOHN

(WITH PORTRAIT)

The untimely death of AARON AARONSOHN in an aeroplane accident occurred May 15, 1919. He was on the postal plane from London, and the plane was wrecked in a heavy fog near Boulogne.



AARONSOHN is best known as the discoverer of wild wheat in Palestine, but his training and enthusiasm were expressing themselves in many ways, not only for the benefit of his own people, but also in helping to solve the general problem of food production. He was born in Roumania very shortly before his parents migrated to Palestine. He was educated in France, and then devoted himself to the agricultural problems and other interests of his home country. His discovery of wild wheat in 1906 was not an accident, but the result of a study of the problem, by which he became convinced that wild wheat would resemble cultivated wheat in appearance and size of grain. He found it growing in rock crevices, and later discovered that it is a relatively common grass throughout Palestine. A drought-resistant and disease-resistant race of wheat fired the imagination of AARONSOHN as to the possibilities of food production. His visits to the United States will always be remembered by those who met him personally or heard his addresses. He traveled throughout the country, investigating the agricultural

conditions, especially in the dry regions, and was convinced that the Palestine wheat would be of great service. Accordingly, in 1909, through the financial support of certain leading Jews in America, there was established in Palestine, at Haifa, the Jewish Agricultural Experiment Station; and in collaboration with the Department of Agriculture the breeding of suitable races of wheat was undertaken. The work of the Station dealt not only with cereals, but also with fruits, and was progressing with remarkable success when it was stopped by the war.

During the war AARONSOHN was absorbed by various activities, and he played an important part in taking General ALLANBY's command across the desert into Palestine, and much of ALLANBY'S success was due to AARONSOHN'S advice and knowledge of the people and of the condition of the country.

In AARONSOHN'S death, at the age of 42, the science of plant-breeding, especially in its practical application in semiarid regions, has probably lost its most promising investigator.—J. M. C.

CURRENT LITERATURE

BOOK REVIEWS

Manual of American grape-growing

This book by HEDRICK[1] belongs to the Rural Manual Series edited by L. H. BAILEY, and, like the rest of the series, aims to cover the field outlined in an up-to-date way. The book is intended to furnish practical working directions to the grower of grapes, whether he uses the native varieties of the eastern regions or the *vinifera* types of the West. The economics of the crop, as well as considerations of soil requirement, vineyard management, and disease control, are presented with clearness, precision, and even with charm of style.

The chapters on breeding and domestication are given increased interest by a brief but comprehensive reference to the very unusual history of grapes and grape-growing in America. The general reader is likely to be surprised to learn how many of our standard varieties have been simply transplanted from the places where they were originally found as mutants or very favorable variations from type. The Concord, Catawba, Scuppernong, Herbemont, Isabella, and a host of other varieties are such products of unaided nature. The hard road traveled by those who in the early times strove to establish the European kinds in the East is lightly indicated. It would hardly be possible in a work of this character to deal more fully with the almost romantic, almost tragic, history of the early pioneers in grape culture. The several determined attempts to grow the European grape financed by various wine companies formed by public spirited men, by colonial governments, and by experienced refugees successful in the wine-growing regions of Europe were all doomed to lingering death, whether located in Pennsylvania, Virginia, or Kentucky. The full story of this phase of our grape history is still to be written.

The taxonomist may be disappointed to find the chapter on "Grape botany" limited to the 11 horticulturally significant species. The author, however, is probably right in tacitly considering the relations in the genus as a whole to be the property of the specialist in plant classification, although he complains of the hazy ideas of those who have thus far dealt with the group.

The book is up to the standard in its mechanical execution. It is abundantly supplied with fine half-tone plates of the most important varieties.

[1] HEDRICK, U. P., Manual of American grape-growing. New York: Macmillan Co. 1919. $2.50.

One may doubt, however, whether the cuts protraying the features of Prince, Rogers, and Munson flatter these leaders. As a whole, the book seems to do well what it is intended to do.—Rodney H. True.

NOTES FOR STUDENTS

Cactaceae.—It is probable that no monograph of a family is based upon more complete study than the monograph of Cactaceae by Britton and Rose,[2] the first volume of which has just appeared. The illustrations are very numerous and admirable, and the colored plates are especially noteworthy. The authors began the study in 1904, and since that time the field work extended beyond North America, which was the original limit, so as to include the arid regions of South America as well. Those who are acquainted with the Cactaceae realize that not only are herbarium and field studies necessary, but also greenhouse studies, to discover the different phases that may appear during development.

The present volume includes the tribe Pereskieae, with its single genus *Pereskia*, represented by 19 species, 4 of which are new, and also the tribe Opuntieae, in which 7 genera are recognized, one of which is new (*Tacinga*). The large genus is *Opuntia*, with 264 species, grouped into 3 subgenera and 46 series, 32 of the species being new. New species are also described in *Pterocactus* (3) and *Nopalea* (2). All of the genera are illustrated, and of the 312 species 267 are represented by one or more illustrations. Of the 36 plates, 28 are in color.

The investigation has been financed chiefly by the Carnegie Institution of Washington, in cooperation with the New York Botanical Garden and the United States National Museum, while the United States Department of Agriculture has taken care of the living collections brought together in Washington. The completed monograph will consist of four volumes.—J. M. C.

Zinc and growth of Aspergillus niger.—Steinberg[3] finds that he gets maximum growth in cultures of *Aspergillus niger* in flasks of Jena glass without additions of zinc sulphate, while less than half maximum growth is given in pyrex and Kavalier Bohemian glass flasks without zinc additions, and maximum growth with such additions (10 mg. Zn/L). Steinberg thinks this is explained by the fact that Jena glass contains considerable zinc, while the other glasses do not. This accords with analysis of Jena and Kavalier Bohemian glass, and with the experiments of other workers on the relation of zinc to the development of this organism. He has also worked with 2 strains of *A. niger*, which he terms *W* and *Y*, and finds that the former demands a higher concentration

[2] Britton, N. L., and Rose, J. N., The Cactaceae. Publ. Carnegie Inst. Washington 1: pp. 236. *pls. 36. figs. 302.* 1919.

[3] Steinberg, R. A., A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. I. The effect of the presence of zinc in the cultural flasks. Mem. Torr. Bot. Club 17:287–293. 1918.

of zinc for its maximum growth than does the latter. He believes that the variation in zinc optimum found by different workers for this species can be explained in part by the difference in the strains, and in part by the difference in the composition of the cultural vessels used. He thinks that pyrex glass, if free from zinc, may bear other substances that stimulate slightly, and that gradual dissolution of these from the glass may account for the continual decrease in yield when cultures are repeated many times in the same flasks. He also admits that other unknown factors may account for this. He thinks it probable that this species has never been grown in total absence of zinc.—Wm. Crocker.

Multiple eggs in bryophytes.—Florin,[4] studying the archegonium of *Riccardia pinguis*, finds the axial row very variable. One archegonium contained an axial row of 4 cells, all of which had developed into eggs; another contained a single egg, 2 ventral canal cells, and 2 rows of neck canal cells; still another contained 4 eggs in the venter after the canal cells had completely disintegrated. Such so-called abnormalities are frequent in bryophytes, making it increasingly clear that both the antheridium and the archegonium are derived from a common gametangium, and that the archegonium occasionally reverts to that time when multiple eggs were the rule instead of the exception. Some mosses revert to a time still more distant, a time when both male and female gametes were present in the same gametangium, since we occasionally find both spermatogenous and oogenous cells in the same sex organ, which usually has the external form of an archegonium.—W. J. G. Land.

Tyrosinase of fungi.—Dodge[5] has made a very careful chemical study of the action of tyrosinase on tyrosin. He obtained his enzyme from *Daedalis confragosa*, *Armillaria mellea*, and *Polyporus sulphureus*. He finds (1) that the tyrosin molecule is not deaminized, and (2) that in the formation of the colored compounds the tyrosin molecules are combined into larger molecules, accompanied by the masking of the carboxyl groups.—J. J. Willaman.

Absorption of gold.—The ability of *Penicillium glaucum* and *Oidium lactis* to develop from conidia in colloidal gold solutions to which tannic acid or gum arabic has been added has been studied by Miss Williams.[6] The colloidal gold is slowly removed from solution during growth, removal being effected by the uncuticularized walls. The gold did not enter the protoplasm. No satisfactory explanation of the phenomena was found.—C. A. Shull.

---

[4] Florin, Rudolf, Das Archegonium der *Riccardia pinguis* (L) B.Gr. Svensk. Bot. Tidskr. 12:464–470. *figs. 4.* 1918.

[5] Dodge, C. W., Tyrosin in the fungi: chemistry and methods of studying the tyrosinase reaction. Ann. Mo. Bot. Gard. 6:71–92. 1919.

[6] Williams, Maud, Absorption of gold from colloidal solution by fungi. Ann. Botany 32:531–534. 1918.

BACK VOLUMES

OF

The Botanical Gazette

AT REDUCED PRICES

This is the time to secure back numbers of the *Botanical Gazette.*

Delay may mean failure to complete your set as the numbers are becoming more difficult to find, while a complete set is rare.

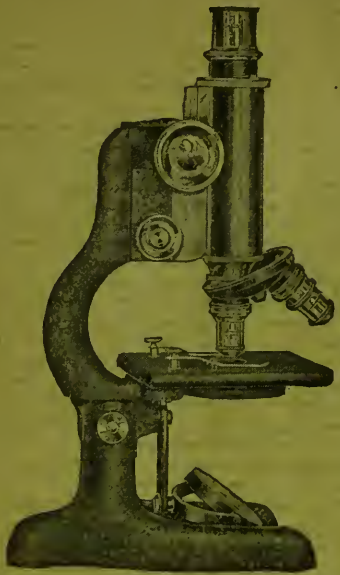
OUR OFFER

Until December 31, 1919, we will sell a limited number of back volumes and single copies at a reduction of 25 per cent from the published prices.

SEND US YOUR ORDER NOW

We have also files of the *Astrophysical Journal* and the *Journal of Geology.*

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - ILLINOIS



FS2 Model with two objectives in revolving nosepiece and one in eyepiece $53.25

Bausch and Lomb Microscopes

are again in the market—backed by the prestige which attaches to successful war service in any significant capacity.

Our release from this service is of course gradual. As the demands made upon us by the government are lessened from month to month, however, we are enabled to increase deliveries to our other patrons.

Write for complete catalog of our revised microscope line and inform us regarding your requirements.

Bausch & Lomb Optical Co.
412 ST. PAUL STREET, ROCHESTER, N.Y.
New York Washington Chicago San Francisco

Leading American Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

FINE INKS AND ADHESIVES
For those who KNOW

Higgins'

- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

At Dealers Generally

CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street Brooklyn, N.Y.

LOOK

for the five alarm clocks that wake up sleepy typewriting. You will find them in the five red keys of the

Self-Starting Remington Typewriter

This machine puts more "go" into your correspondence and insures an increased typing output in the same typing time. Let us send our salesman to tell you more about it.

Remington Typewriter Company
Incorporated
374 Broadway, New York

Volume LXVIII Number 6

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

DECEMBER 1919

Relative Transpiration of Coniferous and Broad-leaved Trees in Autumn and Winter - - - - - J. E. Weaver and A. Mogensen 393
(With eighteen figures)

Torsion Studies in Twining Plants - - - - - H. V. Hendricks 425
(With six figures)

Early Development of Floral Organs and Embryonic Structures of Scrophularia marylandica - - - - - F. M. Schertz 441
(With Plates XXVII–XXIX)

Companion Cells in Bast of Gnetum and Angiosperms - W. P. Thompson 451
(With seven figures)

Secretion of Amylase by Plant Roots - - L. Knudson and R. S. Smith 460
(With two figures)

Ray Tracheid Structure in Second Growth Sequoia washingtoniana H. C. Belyea 467
(With five figures)

Perithecia with an Interascicular Pseudoparenchyma - F. L. Stevens 474
(With Plate XXX)

Current Literature
Book Reviews - - - - - - - - - - - 477
Notes for Students - - - - - - - - - - 480

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

Volume LXVIII Number 6

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued December 20, 1919

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $7.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $7.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $7.84), on single copies 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4. Yearly subscriptions, including postage, 36*s.* each; single copies, including postage, 4*s.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 15.75 each; single copies, including postage, Yen 1.75 each.

For China: The Mission Book Company, 13 North Szechuen Road, Shanghai. Yearly subscriptions, $7.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 84 cents, on single copies 11 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

Illustrations are furnished without cost to author only when suitable originals are supplied. A copy of the suggestions made in the January number, 1907, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

THE
BOTANICAL GAZETTE

DECEMBER 1919

RELATIVE TRANSPIRATION OF CONIFEROUS AND BROAD-LEAVED TREES IN AUTUMN AND WINTER[1]

J. E. WEAVER AND A. MOGENSEN

(WITH EIGHTEEN FIGURES)

Introduction

Transpiration has been of special interest to many investigators for a long time. At first it was considered without reference to environmental factors, but later, as more observations were made and these factors were noted to have a marked effect upon the water loss, they were taken into consideration. Many of the data assembled have been limited to plants during the growing season, so that it has seemed profitable to obtain not only quantitative data on winter losses, but also a comparison of the relative transpiration of conifers and broad-leaved trees in summer and winter.

Various methods have been devised for determining transpiration, from the cut shoot potometer, which usually gives losses quite too low when compared with rooted plants (**6, 16**), to the cobalt chloride method of STAHL (**17**), recently improved by LIVINGSTON and SHREVE (**13**). With few notable exceptions, such as the method used by ILJIN (**11**), who worked on xerophytes and mesophytes in the field, the former method has been used largely for laboratory measurements, while the latter, although especially devised for field use, does not take into account the environmental

[1] Contribution from the Department of Botany, University of Nebraska, new series, no. 29.

factors of the habitat under which the plants are growing except as measured by the internal transpiring power of the plant, nor does it give a record of the continuous transpiration losses.

Unquestionably the most reliable data have been those obtained by the potometer, where the entire sealed container was weighed with the whole plant intact. A survey of the literature, however, reveals rather scant data on the transpiring power of trees, especially coniferous trees, regardless of the method employed.

That evergreen trees are constantly supplied with water, even in winter, was first observed by HALES (8), and later by DUHAMEL (4), TREVIRANUS (18), and others.

In 1860 HARTIG (10) made some investigations on transpiration losses with *Picea*, a meter high, in milder winter, and found that the plant lost from about 100–125 gm. of water a day. These figures, however, are of little value so far as calculating the intensity of transpiration is concerned, since he gave neither area nor weight of the transpiring part.

BURGERSTEIN (3) in 1875 indicated the relation of transpiration to lower temperatures, and showed that cut branches of *Taxus baccata* transpired in an hour, at −2° C., 0.288 per cent, and at −10.7° C., 0.019 per cent of their fresh weight.

That transpiration may take place quite rapidly at rather low temperatures has been shown by WIESNER and PACHER (22). Twigs of *Aesculus* and *Quercus*, for example, lost 0.32 and 0.25 per cent respectively of their weight in 24 hours at −3.5° to −10.5° C., and 0.199 and 0.192 per cent at −5.5° C. to −13.0° C.

BEACH and ALLEN (1) found a loss of from 4 to 9 per cent of water in apple twigs during a single week in January, with a minimum temperature of −26.0° C. They found also in general that the hardiest varieties were the most resistant to water loss.

According to WARMING (20, p. 310), coniferous trees exhale much less water vapor than dicotyledonous trees, due to their xerophytic nature.

KUSANO (12) has given convincing quantitative data on the transpiration of evergreen trees indigenous to Japan. He found that evergreen trees transpired in winter an average quantity of at least 0.48 gm. per sq. dm. per day (with the exception of conifers),

or 16.58 gm. per 100 gm. of fresh weight in foliage trees, and 8.18 gm. in conifers. He also noted that the time of minimum transpiration agrees with that of the minimum temperature, which occurred at the end of January. He states further that the difference in the amount of transpiration of different species of evergreen trees becomes smallest at the time of minimum transpiration; and a change in the external conditions, especially in temperature, does not necessarily produce a corresponding change in transpiration in different species. In average cases the amount of water transpired by foliage evergreen trees is one and a half or two times greater than that transpired by conifers if we reduce the amount either to the fresh weight or to the dry weight of the transpiring part.

VON HÖHNEL (19) estimated that a birch tree, with about 200,000 leaves and standing perfectly free, would evaporate 400 liters of water on a hot dry day. He also has calculated that during the period of vegetation the beech requires 75 liters, and the pine only 7 liters for every 100 gm. of leaf substance. The same writer gives us the following table on the relative amount of water transpired from June 1 to November 30 per 100 gm. dry weight of leaf:

Birch	67.9	Oak	28.3
Lime	61.5	Red spruce	5.8
Beech	56.6	White pine	5.8
Maple	46.2	Silver fir	4.4
Elm	40.7	Austrian pine	3.2

Experiments on the transpiration of seedlings of *Acer saccharinum* in prairie and shrub thicket have been carried out by WEAVER and THIEL (21). Trees placed in the latter habitat lost only 30 per cent as much water per sq. dm. as those in the prairie. Similar experiments with *Quercus macrocarpa* gave comparable results. The high transpiration losses in the prairie help to explain the absence of trees from such localities.

BERGEN (2) compared the transpiration rates of a number of broad-leaved evergreens, including *Olea europaea*, *Quercus Ilex*, and *Pistacia Lentiscus*, with those of equal leaf surfaces of *Ulmus campestris* and *Pisum sativum*. He found that the losses in the

former group were only about 25 per cent less than in the latter, and concluded "that xerophytic leaf structure is not always incompatible with abundant transpiration, but sometimes exists only for use in emergencies to protect the plant from injurious loss of water."

HANSON (9) has shown that the major water losses from the crowns of isolated trees of *Ulmus americana*, *Acer saccharinum*, and *Fraxinus lanceolata* occur from the peripheral branches, and less than one-sixth from equal areas of leaf surface on shaded branches.

The present investigation was undertaken with a double purpose of obtaining data on the relative losses in summer and winter of conifers and broad-leaves, and also to make a beginning on the problem of winter killing of trees and shrubs. This latter project is not included in this paper.

Methods.

In the spring of 1918 seedling conifers of *Pinus ponderosa* Dougl. and *Pinus Banksiana* Lamb. were obtained from the forest nursery at Halsey, Nebraska; while those of *Abies grandis* Lindl., *Pinus Murrayana* Balf., *Picea Engelmanni* (Parry) Engelm., and *Pseudotsuga mucronata* (Raf.) Sudw. were secured from the national forests of northern Idaho. These seedlings, varying from two to four years in age, were potted during May in 5-, 7-, and 8-inch pots respectively, according to the size of the plants and the demands of the root systems, and in soil consisting of two parts of rich garden loam and one part of sand, thoroughly mixed and screened through a one-fourth-inch-mesh sieve. The pots were placed on the lawn near the greenhouse until needed in late summer, and were thoroughly watered every day and sometimes twice a day during the driest periods. A few trees, mostly white fir and Engelmann spruce, died. These, with numerous weaker individuals of other species, were discarded, and only the very best plants, which showed the most flourishing condition of growth, were used in the experiments; in fact, only about half of the original stock was thus selected and used.

Galvanized iron containers with flat bottoms and straight walls were used in the transpiration work, the size varying according to

the demands of the root system. Thus, while the 2-year-old Engelmann spruce required containers only 6.5 inches deep by 3.5 inches wide (these were the smallest used), the 3-year-old yellow pines were grown during the experiments in containers 5.5 inches in diameter and 14 inches deep. Such containers are very desirable, since they combine lightness with appropriate shape for using the minimum amount of soil.

In September, when the trees were transplanted from the pots to the galvanized iron containers, they were handled in such a way as to scarcely disturb the root systems. A layer of coarse gravel 0.25 inches deep was placed in the bottom of each container, to the side of which, extending from the bottom to the top, a heavy glass tube 5 mm. in diameter was fastened with sealing wax. The soil in the flower pots, having been well watered 24 hours previous to transplanting, and the water allowed to drain through the bottom of the pot, was of such a texture that the whole contents could easily be removed by inverting the pot and jarring the edge while holding the soil surface intact with a piece of cloth. This core of soil, containing the root system practically undisturbed, was placed in the new container. In some cases it was necessary to trim away a part of the top of the conical core, but the part removed was always free from roots. Previously the sheet metal container had been filled to such a depth with soil of the same composition as that of the core, that the plant, when put in place, would be at a proper height in relation to the top of the pot. Soil samples for moisture content determinations were taken at this time. Any spaces in the new containers were carefully filled with soil which was properly compacted. Several plants had their root systems more or less disturbed in repotting, and these were discarded. Finally, the pots were sealed.

The seals consisted of petrolatum mixed with paraffin; the latter had a melting point of about 50° C. Various mixtures were used, from 80 per cent paraffin and 20 per cent petrolatum (by weight) at the beginning of the experiment, when the weather was hot, to 25 per cent paraffin and 75 per cent petrolatum in midwinter. A very satisfactory seal for winter weather consists of the latter mixture poured on the surface of the soil while very hot, and then

covered with a less plastic seal of about equal parts of the two ingredients. Little difficulty was experienced in keeping a perfect seal intact.

The broad-leaved trees, *Acer saccharinum* L., *Ulmus americana* L., and *Quercus macrocarpa* Michx., were handled in identically the same manner as the preceding, except that they were grown from seed sowed in flats in the greenhouse, but the plants were transferred to pots out of doors in June.

In order to insure uniform soil temperature conditions for all plants concerned, and changes of temperature similar to those under

FIG. 1.—Arrangement of trees in containers, with collars to insure temperature changes comparable with those of plants growing under natural conditions.

natural conditions, the containers (approximately 100 in number) were placed in tin cylindrical collars, each collar being slightly larger than its respective container. These were in rows about 6–10 inches apart and completely surrounded by soil. The top of each container and collar was covered with a heavy woolen blanket, slit to accommodate the stem, and this in turn with a piece of thick muslin waterproofed by infiltration with the hot wax seal mixture. The edges especially, and also the whole cloth, as well as the surrounding soil, were covered with a thin layer of sand. To prevent the drifting of snow among the trees thus planted on the University campus, the collars and inclosed containers were set on the bare soil surface, and the well-tamped soil filled in between them was held in place by a board frame 12 inches high (fig. 1.)

Continuous records of the humidity of the air and the temperature of both air and soil were obtained by means of Friez's hygrothermographs. The last was for a soil depth of 6 inches among the containers. A thermometer placed at a similar depth in one of the medium sized containers, and protected from external atmospheric conditions by means of a felt-lined brass case fitted with a cap, gave readings very similar to those of the soil, as may be seen in table I.

TABLE I

SOIL TEMPERATURE AT A DEPTH OF 6 INCHES INSIDE A CONTAINER, AND AT A SIMILAR DEPTH OUTSIDE IN THE SOIL

Date	Temperature among roots	Temperature outside
December 18 A.M.	1.5° C.	1.5° C.
18 P.M.	2.0	2.0
31	−4.0	−4.0
January 1	−2.5	−2.5
2	0.0	0.0
3	1.5	1.0
14	−7.5	−8.0

While the factor data will be discussed in connection with the transpiration graphs, it may be said here that at no time was the soil in the containers frozen solidly to a depth greater than 2.5–3.0 inches, a point extending not far into the root zone.

Transpiration losses were determined by weighing the containers. A large long-armed Troemner balance was used which was sensitive to 0.5 gm. under the maximum load of about 8–9 kg. imposed upon it. In making the weighings the containers were transferred from their place out of doors into a weighing room cooled to nearly a similar temperature, for, as shown by WINKLER (23), leaves of evergreens and twigs of other trees can endure from four to six times as much cold if the changes are gradual as if they are sudden. Unless the water loss was rather insignificant as compared with the amount of soil concerned, the practice followed was to replace the loss at each weighing by adding the proper amount of water from a burette through the temporarily uncorked glass tube in the side of the container. Thus the soil moisture was kept at an almost uniform condition throughout.

At least two or three times during the course of the experiments, which extended from September 1917 to May 1918, the seal was broken and the soil thoroughly aerated by means of an aspirator. An examination of containers discarded from time to time because of accidents to the aerial parts of the plants showed that at all times the soil was sweet and in good condition. Such an examination also revealed that the roots of both deciduous and coniferous trees had penetrated somewhat into the new soil. As pointed out by MacDougal (14), the root growth of broad-leaves corresponds to the warm periods during which absorption is active, while any

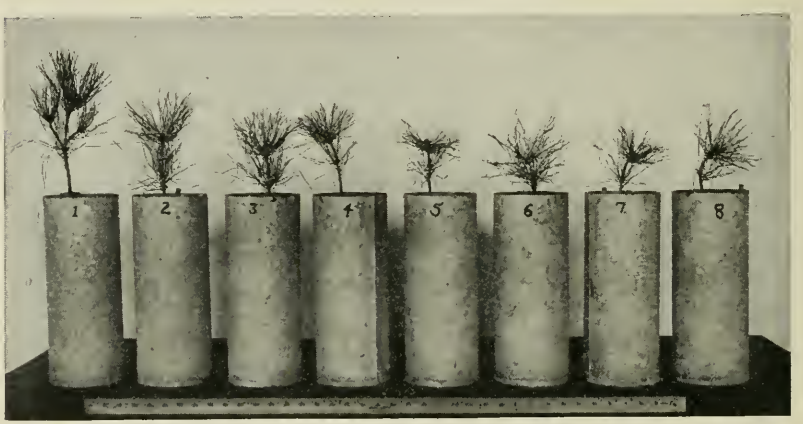


Fig. 2.—Battery of 3-year-old yellow pines; photographed February 28, 1918; none of needles fallen.

possible resting period in summer is deemed due to scarcity of water and not to any inherent tendency of the plant toward a periodicity in growth.

Pinus ponderosa

Eight 3-year-old seedlings of yellow pine were repotted, as already described, into the metal containers 5.5 inches in diameter and 14 inches deep, and the first weighings were made on September 24. This battery is shown in fig. 2. The total leaf area was determined for several of the plants the following spring and at the end of the experiment. This ranged from a minimum of 2.905 sq. dm. in plant no. 7, to a maximum of 6.428 sq. dm. in plant no. 1

(fig. 2). The areas were determined by removing the leaves from the plants, measuring the length of the leaf fascicle and the average diameter of the flat faces, as well as the diameter of the cylinder formed by the two or three leaves in the fascicle when the flat faces were appressed against each other. From these data the actual surface area was calculated. Practically no needles were shed during the winter.

Fig. 3 shows the total average daily losses of six yellow pines from September 24 to January 1. An examination of these graphs reveals a striking similarity. The highest losses are from tree

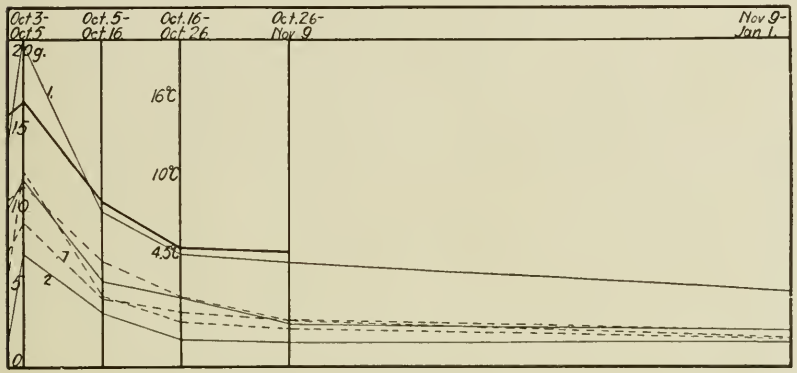


FIG. 3.—Average daily losses in grams from six 3-year-old yellow pines from September 24 to January 1; heavy line represents mean temperature for the several periods.

no. 1 with the greatest leaf area (6.428 sq. dm.), while the lowest losses are plotted from data obtained from tree no. 2, which had an area of only 4.1 sq. dm. Comparative losses per unit area are given elsewhere. Data from the other two pines were omitted in this figure for the sake of clearness. A general relation between temperature and transpiration was clearly evident. The relation to humidity was not so apparent.

The entire period from September 24 to October 16 is characterized by relatively high transpiration losses, after which there is a decided falling off. On October 11 the stomata were found to be closed. The midwinter transpiration losses are exceedingly small. Weighings made on February 7 and after a period of prolonged cold

weather (the mean monthly temperature of January being only 12.6° F.) gave total maximum losses for the entire period of 37 days of only 2.5 gm. The average total loss during this time was about 1 gm. It is surprising how an area of 3–6 sq. dm. of leaf surface can be exposed with such minimum losses. The daily losses, compared with those from the same plants during the period September 24 to October 11, are only 1/251 as great. The average losses during succeeding intervals are shown in table II.

TABLE II

TOTAL AMOUNT (IN GM.) OF WATER TRANSPIRED BY 3-YEAR-OLD YELLOW PINES FROM JANUARY 1 TO MAY 2, 1918

Plant	January 1–February 7 (37 days)	February 7–March 13 (34 days)	March 13–26 (13 days)	March 26–April 29 (34 days)	March 26–May 2 (37 days)
1	2.0	13.7	8.8	7.3	.....
2	0.5	.....	12.2	16.5	.....
3	2.5	9.5	11.0	13.5	.....
4	1.5	8.0	14.5	.....	69.0
5	1.5	7.9	16.1	.....	70.0
6	0.5	10.2	13.8	.....	26.0
7	0.5	4.5	.....	.....	.....
8	0.5	13.1	23.9	.....	91.4

A general but slow rise during the cold month of April may be noted, with a sharp increase following the milder weather in May. The transpiring area throughout is practically constant, since little growth occurred before May 1. On April 3 the stomata were found to be open.

Initial weighings from a battery of five 2-year-old yellow pines, each in a 4×9-inch container, were obtained on October 18. Two of these plants are shown in fig. 12. Table III gives the total losses during the several periods.

On December 19 the leaves were falling so badly that all but two plants were discarded, but the following spring all put out new leaves.

The exceedingly low water losses during the period December 19 to March 13 correspond with those found for the other battery of older yellow pines; in fact, this was found to be the case with all of the conifers. Table III shows an increase in the transpiring

power as spring advanced similar to that of the older pines in the other battery. These plants were growing in a soil with an average water content of 15.8 per cent (18.2 per cent maximum,

TABLE III

TOTAL TRANSPIRATION LOSSES (IN GM.) OF 2-YEAR-OLD YELLOW PINES FROM OCTOBER 18 TO MAY 2

Plant	October 18–November 17 (30 days)	November 17–December 19 (32 days)	December 19–March 13 (84 days)	March 13–April 2 (20 days)	April 2–May 2 (30 days)
1	44.0	12.0	.....	.....	.....
2	48.0	14.1	.....	.....	.....
3	42.0	4.0	.....	.....	.....
4	17.6	6.1	4.3	8.2	13.6
5	22.5	6.5	3.4	6.3	12.5

14.6 per cent minimum), and an available water content varying from 13.5 to 10 per cent.

Pinus Banksiana

A battery of eight 3-year-old jack pines in containers 4.5 inches in diameter and 10 inches deep was sealed and weighed on September 26. These trees were growing in a soil with an actual water content ranging from 8.6 to 15.1 per cent, of which only 4 and 10.3 per cent respectively were available for growth.

The leaf area, calculated in a manner similar to that already described for the yellow pines, ranged from 2.141 to 4.470 sq. dm. The plants remained in good condition throughout the winter and showed vigorous growth in the spring. In addition to the usual brownish color of the leaves in winter, however, the tips of many of the leaves died during January and February. Practically all the leaves remained on the plants throughout the experiment. Fig. 4 shows this battery as it appeared on February 28.

The transpiration losses as determined for the several periods are plotted in fig. 5. A glance at these graphs shows two things which are at once apparent. First, the general parallelism of the lines throughout (except from October 17 to 26, to be considered later); that is, the plant which gave the highest or lowest losses during the early periods continued this behavior throughout. The

actual losses are generally correlated with the leaf area; for example, the greatest losses are from plant no. 7 with an area of 4.47 sq. dm., while those in graph no. 2 are from a plant with an area of only 2.14 sq. dm. Secondly, there may be noted a rise or fall in the graphs which corresponds in general with temperature changes. After October 17 there was a gradual but marked falling off in water

FIG. 4.—Battery of 3-year-old jack pines

losses, and the rate remained very low until about the first week in April, when growth was resumed. These data are shown in table IV.

TABLE IV

TOTAL TRANSPIRATION LOSSES (IN GM.) FROM 3-YEAR-OLD JACK PINES DURING THE SEVERAL INTERVALS FROM DECEMBER 19 TO APRIL 29

Plant	December 19–February 7 (50 days)	December 19–March 26 (97 days)	March 26–April 29 (34 days)
1	.....	39.5	96.4
2	.....	14.0	36.5
3	.....	8.0	48.5
4	.....	24.5	89.9
5	.....	20.2	56.5
6	3.0	February 7–March 26 (47 days)	.....
7	2.0	12.0	.....
8	3.5	30.8	90.0

The midwinter losses, actual and relative, are just as marked as they were in the case of the yellow pines. During the period December 19 to February 7 the total average loss was only 2.8 gm., an amount which, compared on the daily basis, is only 1/169 of that lost during the autumn period (September 26 to October 17).

Abies grandis

Two batteries of white firs were used. One consisted of eight 2-year-old seedlings in containers 3.5 inches in diameter by 6.5 inches deep, and the other of eight 4-year-old trees in containers 3.5 inches in diameter and 9 inches deep.

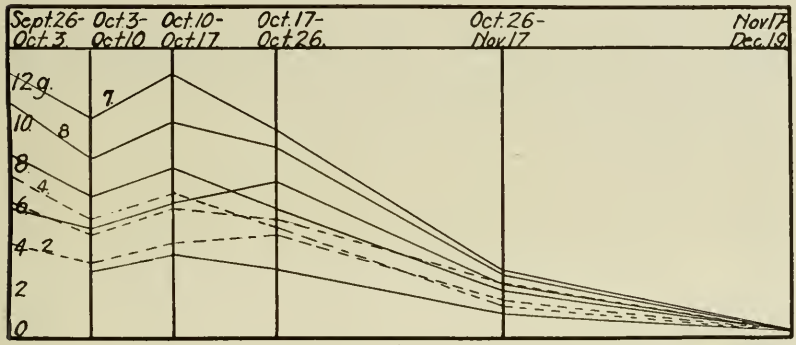


FIG. 5.—Average daily losses in grams from eight 3-year-old jack pines; September 26 to December 19, 1917.

The younger plants were growing in soil with an available water content ranging from 6 to 9.4 per cent. All were winter killed. As this did not occur until January or February, the transpiration losses up to that time are reliable. About January 15 the leaves began to drop off badly, but the leaf area of several of the plants was determined without removing the leaves, and before defoliation had begun. This was accomplished by determining the number of leaves, their lengths, and average diameters. Three of these seedlings had leaf areas of 0.0972, 0.1641, and 0.0970 sq. dm. respectively. These plants are shown in fig. 11. This picture was taken late in February after many of the leaves had fallen. Because of an accident to the top of one tree, it was discarded.

The transpiration losses from September 27 to December 12 from the seven remaining plants are shown in fig. 6. The average total loss during the period of December 12 to February 11 was only 0.7 gm., the maximum and minimum losses being 1.0 and 0.3 gm. respectively. This daily winter loss compared with that of early fall (September 27 to October 10) is only 1/55 as great. It must be noted, however, that it was during this period that the seedlings were winter killed and may have lost more water than

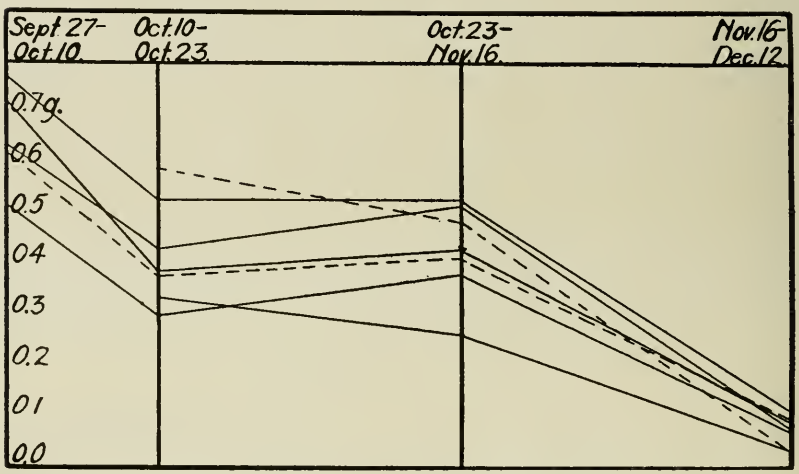


FIG. 6.—Average daily losses in grams from seven 2-year-old white firs from September 27 to December 12, 1917.

normally. On February 11 the leaves were brown and the plants appeared dead.

The 4-year-old firs, although growing under more favorable moisture conditions (from 5.3 to 13.7 per cent available moisture), also succumbed to the dry cold winter. On February 11 the leaves were all brown and dead. The transpiration losses to December 19 are reliable, however, for up to this time all the plants were in good condition. The leaves did not drop badly, even after drying, as is shown in fig. 7, a photograph which was taken on February 28. Leaf areas for plants 5 and 6 were calculated as for the other white firs. These leaf areas were 1.065 and 0.638 sq. dm. respectively.

Since weighings of both sets of firs were taken on the same days, the graphs in fig. 6 may be compared directly with those in fig. 8.

FIG. 7.—Battery of 4-year-old white firs; photographed February 28, 1918, after some of leaves had fallen.

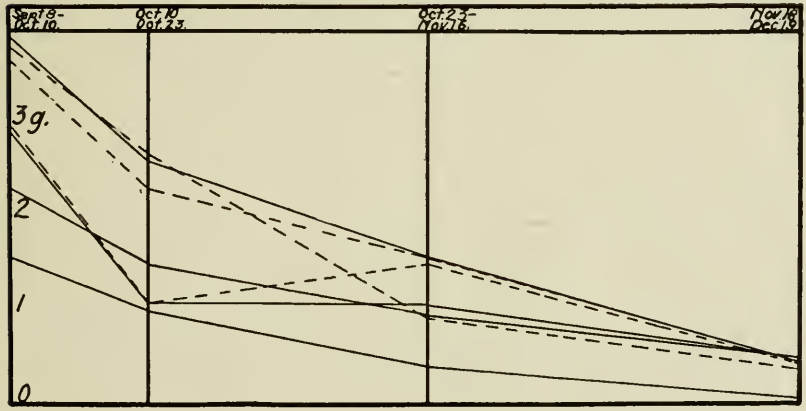


FIG. 8.—Average daily losses in grams from battery of seven 4-year-old white firs; September 28 to December 19, 1917.

A marked irregularity in the course of the otherwise generally parallel graphs may be seen during the period of October 23 to November 16.

Attention has already been called to this phenomenon in case of both the yellow and jack pines, and figs. 9 and 10 show the same occurrence for the spruce and Douglas fir. Although this phenomenon was not examined carefully at the time, for it was not known

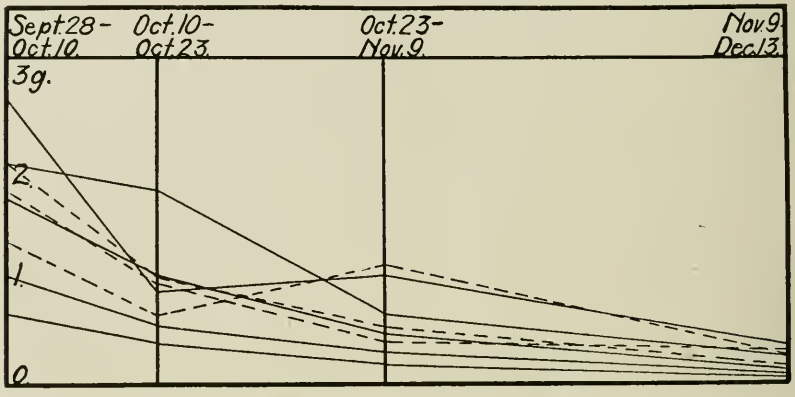


FIG. 9.—Average daily losses in grams from battery of eight 3-year-old spruces; September 28 to December 13, 1917.

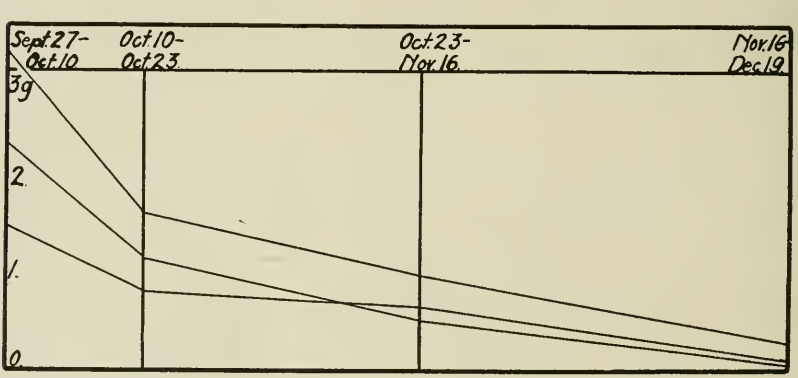


FIG. 10.—Average daily losses in grams from battery of three 3-year-old Douglas firs; September 27 to December 19, 1917.

to occur until after the final weighings on November 16 were plotted, the irregularity is probably due to the individuality shown by the several plants in the rapidity of permanent closure of the stomata, and a general slowing down of the vital activities as the temperature decreased.

Picea Engelmanni

This battery consisted of eight 3-year-old Engelmann spruce seedlings grown in containers 3.5 inches in diameter by 6.5 inches in depth, and in soil with available moisture ranging from 11.3 to 15.1 per cent.

Although the leaves dropped off badly during late December and in January, none of the plants died. All put forth a vigorous growth of new leaves during April and May of the following spring. Three of these trees, photographed in February, are shown in fig. 11.

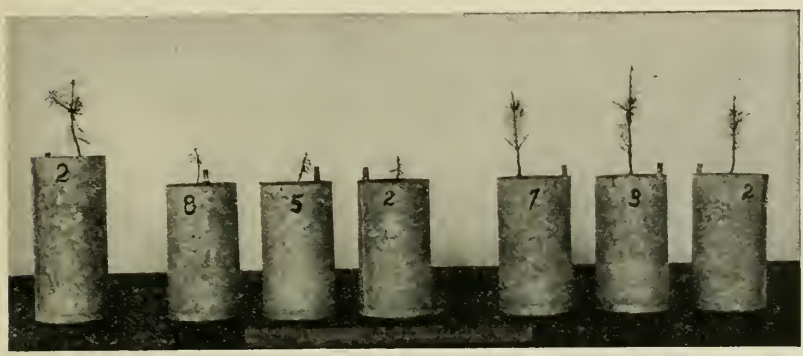


FIG. 11.—Three 3-year-old Engelmann spruces, three 2-year-old white firs, and one Douglas fir, all more or less defoliated; photograph taken February 28, 1918.

The leaf areas of three plants, calculated at the time the leaves had just begun to fall, were 2.78, 3.91, and 5.87 sq. dm. respectively. These areas were determined while the leaves were still intact by counting their total number, measuring the length and average diameter of each leaf, and then calculating the area of the four sides. The graphs giving the transpiration losses are shown in fig. 9. A comparison of the water losses from these plants with those of other conifers shows a remarkable similarity.

Pseudotsuga mucronata

Because of high mortality among the Douglas firs during the reestablishment in pots in early summer, only three 3-year-old seedlings were available for experimental work in September. These were placed in containers 3.5 inches in diameter by 8 inches

in depth, sealed and weighed on September 27. The water content of the soil in the three containers was 13.8, 15, and 18 per cent respectively. The wilting coefficient was 4.7. One of these plants is shown in fig. 11.

The leaf area of plant no. 1 was 0.3062 sq. dm. This was determined by considering the leaves as having two flat surfaces and multiplying the length of each by its average diameter.

Transpiration losses are shown from September 27 to December 19 in fig. 10. During the period December 19 to February 11, the total losses of two plants (the third having accidentally been

FIG. 12.—Battery of five 3-year-old lodge pole pines and two 2-year-old yellow pines; photographed February 28, 1918.

broken off) were 0.4 and 1.1 gm. respectively. On the basis of the average daily loss, this is a mere fraction (only 1/146) of that during the autumn period September 27 to October 10. In March the plants were discarded because of the falling leaves; in fact, one finally died, but the others showed renewed growth in the spring.

Pinus Murrayana

Still another battery of needle-leaved plants, consisting of six 3-year-old lodge pole pines, was experimented upon during the period beginning October 18. These plants, grown in containers 4 inches in diameter and 9 inches in depth, are shown in fig. 12. The water content of the soil ranged from 16.4 to 21.8 per cent.

All of the plants died in February, although the needles persisted for a long time.

The transpiration losses shown in table V are not greatly different in their amount from those of the other conifers. Likewise they show a gradual decrease as winter approaches, with minimum midwinter losses. All losses recorded are reliable because no readings were taken after the plants showed signs of deterioration.

TABLE V

TOTAL LOSSES (IN GM.) FROM A BATTERY OF SIX LODGE POLE PINES FROM OCTOBER 18 TO FEBRUARY 11

Plant	October 18–October 26 (8 days)	October 26–November 17 (22 days)	November 17–January 4 (48 days)	January 4–February 11 (38 days)
1	4.5	9.5	3.9	0.7
2	13.4	30.4	6.0	0.7
3	2.9	15.5	5.5	1.6
4	5.3	13.5	2.6	0.7
5	7.5	15.5	7.8	1.1
6	11.5	37.7	11.3	1.0

Ulmus americana

Initial weighings of a battery of 12 white elm trees were made on September 20. These plants were in containers 5.5 inches in diameter and 8.5 inches deep. A photograph taken on May 1 just when the plants were leafing out is shown in fig. 13.

The leaf areas were determined, as in the case of the other dicotyledons, by means of solio leaf prints. These were made, of course, without removing the leaves from the stems. The areas of the plants whose transpiration losses are shown in fig. 14 were as follows:

No. 6	10.28 sq. dm.	No. 10	4.15 sq. dm.
7	8.57	11	4.52
8	6.56	12	5.26
9	8.97		

For the sake of clarity, the losses from the other plants were not recorded in fig. 14. In all cases they were very similar. With the exception of plant no. 12, there is a close agreement between

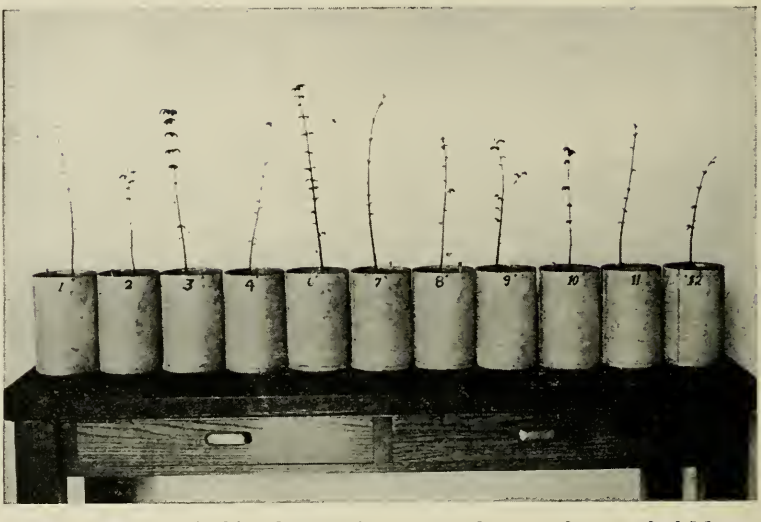


FIG. 13.—Battery of white elms putting out new leaves; photographed May 1

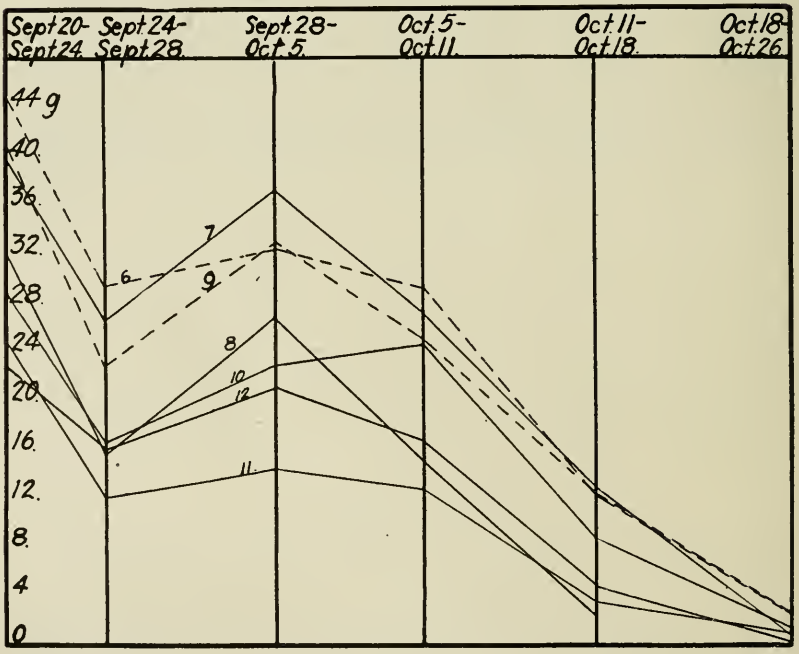


FIG. 14.—Average daily losses in grams from seven 1-year-old elm trees; September 20 to October 26, 1917.

the early autumn losses (September 20 to October 11) and the initial leaf area. Also a comparison of the graph of mean temperature for the several periods (fig. 15), with the graphs showing the march of transpiration, shows that they have a striking resemblance. The mean temperature was obtained from the thermograph records, and is given on a time-humidity basis. The means for the several periods respectively were obtained by drawing a horizontal line through the weekly record sheet in such a manner that the total area included by the graph above this line was equal to the total area below the line. The areas were determined by the aid of a planimeter. In this interpretation both temperature and time factors are taken into consideration. Temperature, indeed, seems to be the controlling factor, for no such correlation between transpiration and humidity (here the graph is inverted) is discernible after October 5; that is, transpiration dropped off at this time, following the descending temperature graph in spite of a decreasing humidity.

These two factors, however, together with wind velocity, are well summed up in evaporimeter readings. These were obtained by Livingston's porous cup atmometers and show an increase or decrease very similar to the transpiration graphs. They are as follows:

September 20–24	25.1 cc. average daily loss
September 24–28	11.8 cc.
September 28–October 6 . .	22.2 cc.

Readings were discontinued on October 11 because of freezing weather at night. Unfortunately no instrument has yet been devised for satisfactorily measuring winter evaporation losses.

The irregularity in the graphs, especially in the fourth interval, is due to the non-uniform drying out of the leaves. Up to October 5 the leaves of all the dicotyledonous plants were green and apparently functioning normally. By October 11 a few of the older basal leaves were beginning to turn brown. While some dropped at once, many held on after they were dry. This irregularity in defoliation defeated an attempt to base the daily losses upon the actual average leaf area during any given period, although an exact

record was kept of the time of the fall of each leaf, together with its area.

The transpiration graphs show a general falling off after October 11, which is not unlike that of the conifers; only here the transpiration is from a constantly decreasing area (all the leaves had fallen or remaining fragments were removed by October 26), while in the conifers the area remains constant. In table VI are given the total losses (which are too small to plot on an average daily basis) from October 26 to May 1.

TABLE VI

TOTAL LOSSES (IN GM.) FROM 12 ELM TREES FROM OCTOBER 26 TO MAY 1

Plant	October 26–November 9 (14 days)	November 23–January 4 (42 days)	January 4–April 24 (110 days)	April 24–May 1 (7 days)
1	.....	1.0	7.4	2.2
2	.....	1.0	5.6	3.2
3	6.9	1.0	12.3	12.7
4	2.8	1.0	6.6	2.7
6	3.3	1.0	11.3	9.7
7	3.4	1.5	10.8	1.3
8	2.0	1.0	5.8	3.2
9	5.7	1.5	9.3	3.7
10	1.9	0.5	7.0	5.8
11	0.4	0.5	4.5	2.3
12	1.5	0.5	7.1	2.2

From November 9 to 23 the seals were removed and the soil allowed to dry out. The loss was about 125–175 gm. They were then resealed, and 250 cc. of water was added. On April 24 some of the plants, as nos. 2, 10, and 11, had buds swollen but not yet open, while in others, as nos. 3, 6, and 7, the leaves were unfolding. On May 1 nos. 3 and 6 had leaves fairly well out, some of them being over 1.5 cm. long, while in others (as nos. 1 and 7) the leaves were just bursting from the buds.

The winter losses are very small. During a period of 42 days (November 23 to January 4) the average loss was only 1 gm. from the bare stems which exposed an area of 0.28 to 0.50 sq. dm. Table IX gives the relative transpiration losses of conifers and broad-leaved trees.

Acer saccharinum

Weighings of a battery of 12 soft maples were made on the same dates as those for the elms. The containers were of the same size,

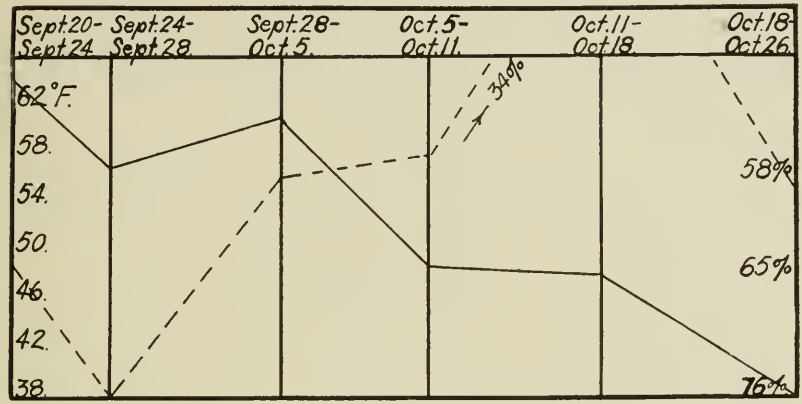


FIG. 15.—Mean temperature (solid line) and mean humidity (broken line) during several intervals from September 20 to October 26, 1917.

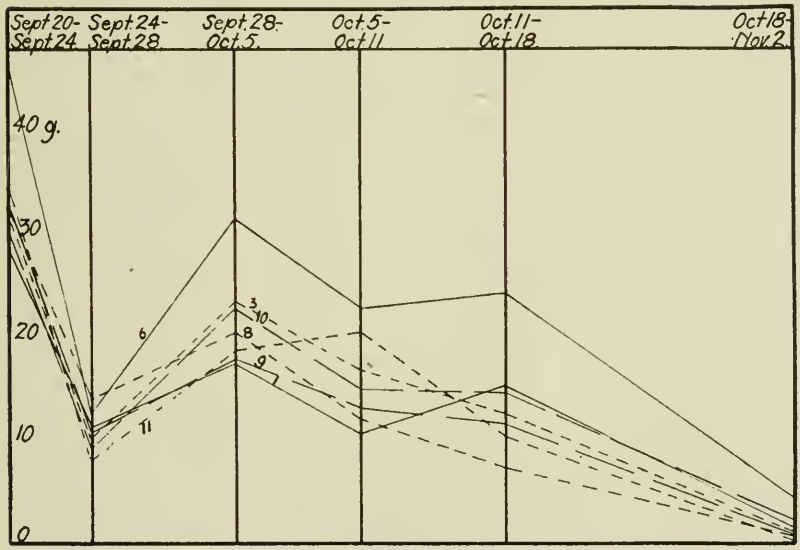


FIG. 16.—Average daily losses in grams from seven 1-year-old maple trees; September 20 to November 2, 1917.

and the batteries and the methods of handling them were so similar throughout that further description will be unnecessary.

The leaf areas ranged from 11.32 sq. dm. in plant no. 6, which gave the highest losses throughout (fig. 16), to 6.47 sq. dm. in no. 11, where the transpiration losses are among the lowest. A good correlation existed between leaf area and the magnitude of the losses. To avoid confusing a multiplicity of lines, data from five of the maples have been omitted in this figure. The data of these five trees are very similar to those shown.

TABLE VII

TOTAL LOSSES (IN GM.) FROM 12 MAPLE TREES FROM NOVEMBER 2 TO MAY 1

Plant	November 2–16* (12 days)	November 28–January 1 (34 days)	January 1–April 24 (113 days)	April 24–May 1 (7 days)	Condition on on May 1
2.....	2.2	0.8	2.9	3.6	Two buds unfolding leaves
6.....	2.5	0.8	4.5	2.2	Just showing signs of life
8.....	3.0	1.1	.....	.....	.....
10.....	1.7	0.3	3.6	70.1	Leaves 1–5 cm. long
12.....	1.8	0.5	2.3	13.5	Two buds with leaves 2 cm. long
	December 6–January 1 (26 days)				
1.....	1.8	.....	.....	.....	.....
3.....	2.0	.....	3.7	4.0	One bud swollen; two others partly open but frozen
	November 2–16* (14 days)				
5.....	1.5	0.9	.....	.....	.....
7.....	2.0	1.8	.....	.....	.....
9.....	1.5	1.0	.....	.....	.....
11.....	2.5	0.5	.....	.....	.....

* All leaves off.

As in the case of the elms, a close correlation exists between transpiration, evaporation, and temperature (fig. 15); in fact, the general trend of the graphs is strikingly similar to those of the elms. The same irregularity is shown during the period of defoliation. On October 26 only the bare stems remained; these had a surface area of 0.22–0.48 sq. dm.

Further losses from November 2 to May 1 are given in table VII. The small losses are again evident, followed by a rapid rise

accompanying the unfolding of the buds. Six plants were winter killed, and no losses were recorded for them after January 1.

Quercus macrocarpa

A number of bur oak seedlings were grown from acorns obtained from an Illinois nursery in May 1917. Seven of the lot were finally selected for experimental work. These were repotted in containers 5 inches in diameter and 7 inches deep. They may be seen in the foreground in fig. 1. The initial available water content of the soil ranged from 11.3 to 15.2 per cent, but the soil moisture was reduced by evaporation from the surface of the several containers

TABLE VIII

AVERAGE DAILY LOSSES (IN GM.) OF BUR OAK SEEDLINGS FROM SEPTEMBER 24 TO JANUARY 4

Plant	September 24–October 3 (9 days)	October 3–10 (7 days)	October 10–18 (8 days)	November 1–16* (15 days)	December 7–January 4 (28 days)
1.....	9.0	7.0	2.1	0.25	1.0
2.....	.....	11.0	4.9	0.55	1.3
3.....	10.2	6.4	4.3	1.22	1.4
4.....	15.5	9.9	1.1	0.26	1.8
5.....	7.2	3.4	0.4	0.21	1.0
6.....	4.5	2.6	0.5	0.28	1.0
7.....	15.2	12.9	8.0	1.89	1.8

* At this time all the leaves had fallen, and the losses are not calculated on the average daily basis, but on the total loss for the entire period.

to a minimum of 4.4 and a maximum of 9.6 per cent respectively from October 18 to November 1. After this the seals were again replaced and half of the containers brought back to the original water content. The transpiration losses are shown in table VIII.

The gradual falling off in the transpiration rate without any increase, as in the case of the elms and maples, may be explained by an examination of the dates, which show that the first loss was not recorded until a time (October 3) when all the trees show a steady decrease. By November 1 all of the leaves had fallen (or the dried leaves were removed). Following this the losses are extremely small. The stem areas of the oaks were only 0.039–0.088 sq. dm.

Relative transpiration rates

Thus far we have considered largely transpiration losses of different individuals of the batteries of the various species, with only occasional reference to comparative losses of different species. It may be well, therefore, to present data showing the actual losses of different species based upon unit area. These data have been summarized in table IX. While the dates between readings are not synchronous in all cases, they are at least nearly so, and fig. 15,

TABLE IX

COMPARATIVE TRANSPIRATION LOSSES FROM UNIT AREAS OF SURFACE OF BROAD-LEAVED AND EVERGREEN TREES

Species	No. of trees	Time	Average loss per sq. dm. per day
Acer saccharinum	9	September 24–October 5	2.66
Ulmus americana	9	" 24– " 5	3.56
Quercus macrocarpa	7	" 24– " 3	5.18
Picea Engelmanni	3	" 28– " 10	4.18
Abies grandis (2 years old)	3	" 27– " 10	5.44
Abies grandis (3 years old)	2	" 28– " 11	4.76
Pinus ponderosa	3	" 24– " 5	4.20
Pinus Banksiana	3	" 26– " 10	4.80

which gives the temperature and humidity, together with the record from the atmometers, shows that the overlapping periods are quite uniform.

Relative midsummer transpiration rates of broad-leaved trees and conifers

During the following summer, while the writers were carrying on other investigations in the Rocky Mountains of the Pike's Peak region of Colorado, opportunity was afforded to compare the summer transpiration rates of different tree species.

Seedlings of white elm and soft maple grown from seed at the Alpine Laboratory near Manitou, Colorado, and at an altitude of 8000 ft., together with mountain maple (*Acer glabrum* Torr.) obtained from the forest, were the broad-leaved species used. Yellow pine, Douglas fir, and Engelmann spruce were the conifers employed. The pines were secured from the Fremont Experiment Station; while the other trees, like the mountain maples, were

transplanted into appropriate sized containers directly from the forest floor, without disturbing the root system. Care was taken

FIG. 17.—Battery of Douglas firs and Engelmann spruces from which summer transpiration losses were obtained.

FIG. 18.—Battery of yellow pines and mountain maples used for determining relative summer transpiration.

not to select these species from too dense shade. Some of these trees are shown in figs. 17 and 18.

The extra soil necessary to fill these containers was in all cases a mixture of two parts of garden loam and one part of fine gravel. It had a wilting coefficient of 10.7 per cent, and was kept rather uniformly at a water content of about 20 per cent. The methods used throughout were the same as those already described. Original weighings were not taken until after the trees had been in the containers two weeks. At this time rabbits attacked the deciduous trees and injured all but five of them.

On July 20 the remaining trees in their containers, 19 in number, were weighed to the nearest half gram. As during the preceding interval, they were then placed in a row in the soil and in such a position that all were shaded for a few hours in the afternoon.

TABLE X

AVERAGE TRANSPIRATION LOSSES PER UNIT AREA FROM SEVERAL SPECIES OF TREE SEEDLINGS

Species	No. of plants	Average loss per sq. dm.
Pinus ponderosa	4	58.2
Pseudotsuga mucronata	5	50.7
Picea Engelmanni	5	58.3
Acer glabrum	4	112.5
Acer saccharinum	1	139.3

At the end of 26 days, on August 15, they were again weighed, the leaf areas determined, and the losses calculated as in the preceding experiments. These data are shown in table X.

These data show that the losses from the various species of conifers, growing in the open but under conditions where all were shaded for a portion of the day, are very similar, and only about half as great as those of the broad-leaved species. The midsummer average daily loss per square decimeter from the yellow pines and Engelmann spruce at the Alpine Laboratory, when compared with that of the same species during the autumn at Lincoln, was found to be only about half as great at the former station. Because of the limited number of plants used, however, these data should be considered indicative rather than conclusive.

Conclusions

A consideration of the foregoing data leads us to some conclusions quite the converse of statements generally current in ecological-physiological literature. Perhaps the most important of these are the facts shown to hold under the conditions of these experiments; first, that broad-leaved trees under late summer conditions have no greater and indeed often a smaller transpiring power, area for area, than conifers; and secondly, that the water losses of coniferous trees during the winter months are relatively no greater with the needles intact than are the losses from deciduous trees after the leaves have fallen.

The fact that all of the plants concerned, including three species and about 30 individuals of broad-leaved trees, and six species represented by about 70 individuals of conifers, gave results which without exception point to these conclusions, leaves little doubt as to the validity of the finds. These results were obtained with all the plants under uniform conditions of soil type and texture, soil temperature, and identical aerial environment. The only variable factor was soil moisture, and here the range was no greater for the one group than for the other. Although the experiments were undertaken near the end of the summer, for a period of a few weeks, after their beginning the deciduous trees were in excellent growing condition, and the comparative losses as here recorded occurred during the earlier part of this period, when the weather was similar to that of midsummer as regards temperature and humidity.

That winter losses from the same leaves that transpired so freely the preceding fall and again in the following spring are so small is certainly testimony of the ecological efficiency of coniferous leaf structure for reducing water losses. Whether this is due entirely to stomatal closure, or, as seems more probable, is connected with chemical changes in cell contents as well, remains to be determined. Such work as that of MIYAKE (15) on the food making of coniferous leaves in winter and EHLERS (5) on temperature is rapidly throwing considerable light upon the winter activities of coniferous trees.

The gradual decline of the transpiring power of broad-leaved trees preceding defoliation and during the formation of the abscisslayer is what might be expected, but is heretofore, we believe, unrecorded. Weather conditions play an important part in hastening or retarding this process, and as a result the dropping off in the transpiration graph. These graphs are surprisingly similar in their general trend to those of conifers where the leaves are intact.

The spring of 1918 was unfavorable for rapid leafing out or renewed growth of trees. Consequently the increased transpiring power, which is in a direct ratio with the leaf area, occurred very gradually, and here again was not greatly unlike that of the needle-leaved trees. Undoubtedly under very favorable weather conditions, as occur during some springs, the transpiring power might easily double or treble day by day for a rather long consecutive period.

Summary

1. Autumn transpiration losses from conifers are just as great as or even greater than those from broad-leaves.

2. The decrease in water losses from broad-leaved trees resulting from defoliation is gradual, and not greatly unlike the decrease shown in the transpiring power of conifers.

3. Winter transpiration losses from conifers are only 1/55–1/251 as great as those in autumn.

4. The increased losses of broad-leaved trees in spring occasioned by foliation are in proportion to the leaf areas exposed, and are closely controlled by weather conditions, but in the main are similar to increased losses of conifers.

5. Winter transpiration losses from conifers are scarcely greater than those from defoliated stems of broad-leaved trees.

The writers are indebted to Dr. R. J. Pool for his kindly interest and encouragement, and to Dr. L. J. Briggs for wilting coefficient determinations; also to Mr. J. Higgins, of Halsey, Nebraska, and Mr. T. J. Larsen, Priest River, Idaho, for tree

seedlings from the nurseries, and to Professor T. J. FITZPATRICK for careful reading of the manuscript and proof.

UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

LITERATURE CITED

1. BEACH, S. A., and ALLEN, F. W., Jr., Hardiness in the apple as correlated with structure and composition. Iowa Agric. Exp. Sta., Research Bull. 21, p. 185. 1915.
2. BERGEN, J. Y., Transpiration of sun leaves and shade leaves of *Olea europaea* and other broad-leaved evergreens. BOT. GAZ. 38:285–296. 1904.
3. BURGERSTEIN, A., Über die Transpiration von *Taxus*-Zweigen bei niederen Temperaturen. Oesterr. Bot. Zeitschr. 25: 1875.
4. DUHAMEL, H. L., De l'exploitation des bois. 1:337. 1764.
5. EHLERS, JOHN H., The temperature of leaves of *Pinus* in winter. Amer. Jour. Bot. 2:32–70. 1915.
6. FREEMAN, GEO. F., Method of determination of transpiration in plants. BOT. GAZ. 46:118–129. 1908.
7. HABERLANDT, G., Physiological plant anatomy, p. 138. Trans. from 4th German ed. by MONTAGU DRUMMOND. London. 1914.
8. HALES, STEPHEN, Statik der Gewachse, p. 29. 1748.
9. HANSON, H. C., Leaf structure as related to environment. Amer. Jour. Bot. 4:533–560. 1917.
10. HARTIG, T., Über die Bewegung des Saftes in den Holzpflanzen. Bot. Zeit. 19:17. 1861; and Lehrbuch für Förster 1:252. 1877.
11. ILJIN, V. S., Relation of transpiration to assimilation in steppe plants. Jour. Ecology 4:65–82. 1916.
12. KUSANO, S., Transpiration of evergreen trees in winter. Jour. Coll. Sci., Imperial Univ. Tokyo 15: part 3. 1901.
13. LIVINGSTON, B. E., and SHREVE, EDITH B., Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World 19:287–309. 1916.
14. MACDOUGALL, W. B., The growth of forest tree roots. Amer. Jour. Bot. 3:384–392. 1916.
15. MIYAKE, K., On the starch of evergreen leaves and its relation to photosynthesis during the winter. BOT. GAZ. 33:321–340. 1902.
16. MUENSCHER, W. L. C., A study of the relation of transpiration to the size and number of stomata. Amer. Jour. Bot. 2:487–504. 1915.
17. STAHL, E., Einige Versuche über Transpiration und Assimilation. Bot. Zeit. 52:117–146. 1894.

18. TREVIRANUS, C. L., Physiologie der Gewäsche. 1:488. 1835.
19. HÖHNEL, FRANZ R. VON, The physiology of plants, by W. PFEFFER, 2d ed., trans. by A. J. EWART. 1:251. 1900.
20. WARMING, EUGENE, Oecology of plants. Oxford. 1909.
21. WEAVER, J. E., and THIEL, A. F., Ecological studies in the tension zone between prairie and woodland. Bot. Survey Neb., conducted by Bot. Seminar, N.S., no. 1, Lincoln. 1917.
22. WIESNER, J., and PACHER, A., Über die Transpiration entlauber Zweige und des Stammes der Rosskastanie. Oesterr. Bot. Zeitschr. no. 5. 1875; cited by HABERLANDT (7, p. 138).
23. WINKLER, ALBERT, Über den Einfluz der Auszenbedingungen auf die Kälteresistenz ausdauernder Gewächse. Jahrb. Wiss. Bot. (Pringsheim) 52:467–506. 1913; abstract in Exper. Sta. Record 30:333. 1914.

TORSION STUDIES IN TWINING PLANTS

H. V. Hendricks

(with six figures)

Introduction

This paper deals with a certain phase of the physiology of twining plants. It is an account of some experiments upon the scarlet runner or flowering bean (*Phaseolus multiflorus*) and the black or corn bindweed (*Tiniaria Convolvulus* [L.] Webb and Moq.), in which a modified form of auxanometer was used. To a limited extent the hop (*Humulus Lupulus* L.) was studied also.

The attention which twining plants have received from many observers has resulted in considerable literature on the subject. The main facts, however, have been well summarized by Vines[1] and by Pfeffer.[2] A résumé of these accounts seems unnecessary for our present purpose, but it is convenient, as well as in accord with the views of these workers, to consider the phenomenon as involving the following as its most striking factors: (1) circumnutation of the growing tip; (2) winding of the stem about its support; (3) torsion of the stem in the same direction as the winding (homodromous); (4) torsion in the opposite direction (antidromous). The relation of these factors to one another is not perfectly understood, and I shall not enter into any discussion regarding it. This paper deals only with torsion of the stem.

Not having ready access to the literature, the aid of Mr. Peter J. Klaphaak, of the University of Michigan, was secured in looking up references, and I desire to express my appreciation of his services. In as extensive a review as it was possible to make, no reference was found to any method of approaching the problem similar to the one here described.

[1] Vines, S. H., Lectures on physiology of plants. 1886.

[2] Pfeffer, W., Physiology of plants, translated by A. J. Ewart, Vol. III. 1906.

Method

The method consisted in stretching vertically a growing internode, or parts of two adjacent internodes, and measuring the amount of twist and the corresponding length. The object of this stretching is, while giving support, to eliminate twining proper, and the amount of stretching is slightly greater than that required to accomplish this. An estimate of the torsional rigidity at different lengths was also made. In certain experiments I have compared this with the diameter and with the amount of lignification. I have also begun studies on antidromous torsion, and have made a few other observations.

The apparatus is illustrated in figs. 1–4. There stands on a base an upright (*Up*) about 1 m. high. This has a top piece bearing two wheels (*W* and *W'*), over which runs a fine silk thread. On the upright are also two sliding clamps (*K* and *K'*), which can be secured at any height. The lower of these (*K*) has an arm bearing a special kind of clamp (*Cl*) for holding the lower part of the growing internode. The other (*K'*) carries a telescope (*T*) and a semicircular scale (*Sc*), which is graduated on the inside so as to read half-degrees. I found 28 cm. a convenient diameter for this scale. The upper part of the internode used is held by the frame (*F*), which is suspended by the silk thread already mentioned. The weight (*Wt*) supplies the proper amount of tension. By means of the scale (*Sc'*), graduated so as to read 0.1 mm., and the double pointer (*I*, *II*) on the wheel (*W'*) the increase in length can be obtained accurately, while this value can be obtained roughly by means of a linear scale hung near the thread on this side of the apparatus, but omitted from the figure for the sake of clearness. There is a curved guard (*G*) to protect the pointers.

The frame (*F*) requires more detailed description. It consists of a bow of heavy wire, 2.2 mm. thick and about 12 cm. in diameter, closed by a yoke (*Yk*) and suspended by a block of wood which holds four mirrors (*M*). These mirrors (fig. 2), each one 1.2 cm. in diameter (or better 1.0 cm.), are arranged in two pairs at right angles, and are held together by means of a piece of sheet metal (best of aluminum), which is cut to resemble roughly a cross and then bent into the form shown. These mirrors are held in place

by fine wires and are labeled *A*, *B*, *C*, *D*. The yoke (*Yk*, fig. 3) is a curved piece of hard wood (about 5×1×0.6 cm.) and has fitted

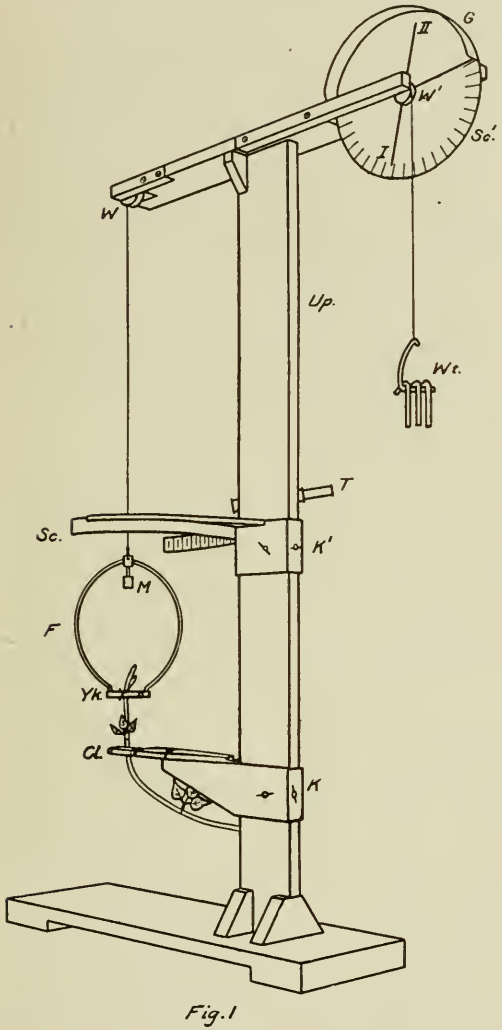


Fig. 1

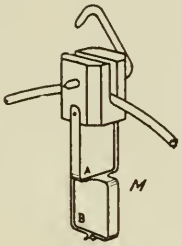


Fig. 2

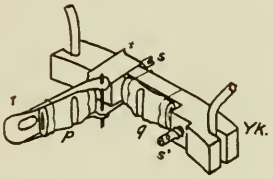


Fig. 3

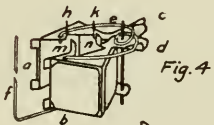


Fig. 4

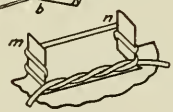


Fig. 4a

H.V.H.

FIGS. 1–4

over it a piece of "tin" (*t*), which is approximately U-shaped, with the "base" of the U back of the yoke. Its position on the yoke can be regulated by means of a peg on the small screw (*s*), only a

little of which can be seen in fig. 3. This peg is between the yoke and the base of the U-piece. Another piece of "tin" (*r*) is hinged upon *t* by a pin, and has fastened to it by fine wire a rubber pad (*p*). Another rubber pad (*q*) is fastened to the yoke. These pads are pieces of 4 mm. rubber tubing (even smaller can be used for some purposes), and each has a groove cut a little more than halfway around it before it is secured in place. A slot in *r* fits over the screw (*s'*) when *r* is closed, and then the grooves on the two pads come together and make a place for holding the stem. A peg through the screw (*s'*) keeps this clamp closed. By adjusting the screws (*s* and *s'*) the correct amount of pressure required to hold the stem from slipping without interfering with its growth is obtained. This is easily determined by a few trials. The entire frame weighs about 20 gm. The clamp for holding the lower part of the stem used is constructed in the same way.

In order to keep tally on the number of complete revolutions which the stem makes in twisting, a black thread (the fine silk thread being white) is attached to the upper part of the bow of the frame, and, allowing plenty of slack, is then carried over a hook near the wheel (*W*). The number of times this is coiled (very loosely) about the silk gives the amount of twist roughly, while it is given exactly by means of the mirrors, telescope, and semicircular scale. For the sake of clearness this black thread also has been omitted from the figure.

One other device which, although imperfect, was found useful in studying intermediate points in the part of the stem used is here described and is illustrated in figs. 4 and 4*a*. I call it a "mirror clamp." It consists of two symmetrical halves (*a* and *b*) cut out of aluminum and bent into shape as shown. These halves are hinged together at one edge, and when closed form essentially a cube without a bottom. A mirror of quite thin glass, about 1 cm. square, is held in place on each side of the cube with bits of adhesive plaster. The clamp is opened by means of holders (*c* and *d*) on the two halves, and it is kept closed by means of the steel spring (*e*). On the top of each half there are two projections (*h*, *k* and *m*, *n*). Fig. 4*a* shows with enlargement how a piece of stout thread is placed on one pair of these. It is not tied, but the ends are twisted

about each other several times (more than shown). On *h*, *k* two loops of this thread are placed, instead of one, and in such a way that the inner part on *m*, *n* comes between the inner parts of the two loops on *h*, *k* when the clamp is closed. A piece of fine copper wire (*f*), several cm. long, is attached to one-half of the clamp. This can be adjusted by bending so as to counterbalance the holders opposite. The entire device weighs somewhat over 1 gm. Since the mirrors are placed at some distance from the vertical axis, slight corrections are necessary for accurate work. In order to measure the growth in length of the point holding this clamp, a linear scale, fastened vertically, was used. With care this measurement could be made with fair accuracy.

In its simplest terms, torsional rigidity may be looked upon as "resistance to twisting." In earlier experiments it was estimated by balancing a measured deflection in the part of the vine used against a measured twist in a very fine wire. As this required additional apparatus, as the readings were rather difficult to make satisfactorily, and as the results were not very accurate, it will not be described in detail. The success of the present method, which is not open to these objections, depends on making the frame light, compact, and with small resistance to the air. In making a determination by the present method, the period of oscillation of the frame is timed. This is, in fact, a common method in physical laboratories. As may be learned from any textbook in physics,[3] the law governing this may be stated as follows: If a circular wire of length L and radius of cross-section R suspends a body whose moment of inertia is K, and if the coefficient of torsional rigidity of the material of this wire is n, then the period of oscillation in seconds of this body is given by the following equation: $T=(1/R^2)\sqrt{8\pi KL\div n}$. From this, $n=8\pi KL\div T^2R^4$. It is more convenient, however, to use the diameter (D) of the vine, and I always use the same frame and am dealing with comparative values only. Consequently, the expression actually used is $L\div T^2D^4$, which equals what may be called *the coefficient of rigidity*. This may be defined for our purposes as *the relative measure of the resistance offered to twisting through a unit angle of an average unit length*

[3] For example, WATSON, W., A text-book of physics. Longmans, Green & Co. 1900. p. 205.

of vine per average unit diameter. As in most of the experiments no accurate measurements of the diameter were made, the expression more commonly used is $L \div T^2$. I shall call this simply *the rigidity*, or *the relative resistance to twisting through a unit angle of an average unit length of the vine.* Certain objections to these terms will be discussed later. As will be seen, they are not constant, but vary.

These experiments were performed in a greenhouse and have extended over several seasons. Generally speaking, conditions were found satisfactory for growth only during May, June, and the first part of July, at least in this particular greenhouse.

The apparatus is set up on a bench and leveled so that the upright is plumb. A weight placed on the base and wires from near the top of the standard to the roof of the greenhouse give the apparatus stability. By means of slots and screws in the top piece and in the arm of the clamp (*K*), adjustments are made so that the vertical line to be taken by the silk and the part of the vine used passes through the center of the semicircular scale. The proper weight is then applied to the silk, and the upper part of the growing internode near the tip is clamped in the frame. The lower part of this internode, or the upper part of the next internode, is then very carefully secured by the clamp (*Cl*).

Measurements of diameter were made by means of a gauge similar to one form of wire gauge. This consists of a pair of straight-edges, one of them graduated, held securely together so as to make a V with a very small angle. Usually many readings were taken (about 10–40), but irregularities in the stem prevented much accuracy. It was not found possible to make many such measurements in the case of the black bindweed. General observations of weather and temperature were recorded, but were found to be of only limited value.

Data and curves

Table I contains in an abridged form the data of a typical experiment. The dates and times of the readings are given with the corresponding lengths and the corresponding amounts of total twist in angular degrees. The average periods of oscillation of the

frame are given. This value, in seconds, is obtained in each case by observing the length of time required for a certain number of oscillations (usually 50 or 100, occasionally 200), making usually 4 or 5 determinations in each case. The average diameters are given. In some cases these were not measured directly, but were obtained from the diameter curve. The calculated values of rigidity ($L \div T^2$) and of coefficient of rigidity ($L \div T^2 D^4$) are also given.

TABLE I

FLOWERING BEAN

No.	Date	Time	Length = L in gm.	Twist in degrees	Period of oscillation in seconds = T	Diameter in mm. = D	D^4	Rigidity = $L \div T^2$	Coefficient of rigidity = $L \div T^2 D^4$
1.....	6–4–18	1:15 P.M.	2.00	0	2.22	1.50	5.06	0.41	0.080
2.....	6–5–18	7:55 A.M.	3.22	62	2.06	1.56	5.93	0.76	0.128
3.....	6–5–18	7:22 P.M.	5.18	391	2.32	1.67*	7.78	0.96	0.124
4.....	6–6–18	11:25 A.M.	7.24	713	2.05	1.80	10.50	1.72	0.164
5.....	6–6–18	6:05 P.M.	8.35	894	1.91	1.85*	11.70	2.29	0.196
6.....	6–7–18	9:25 A.M.	9.58	1078	1.70	1.91*	13.30	3.32	0.250
7.....	6–7–18	8:00 P.M.	10.01	1128	1.00	1.90	13.01	10.01	0.770
8.....	6–8–18	2:57 P.M.	9.92	1108	0.75	.....	.....	17.60	.....
9.....	6–8–18	5:55 P.M.	10.00	1105	0.72	1.92	13.60	19.30	1.419
10.....	6–9–18	7:20 P.M.	10.08	1102	0.65	1.96	14.75	23.86	1.617

* Value obtained from diameter curve: points *a*, *b*, and *c*, fig. 5.

In fig. 5 these data are presented in curves. In all cases abscissae represent lengths. It will be observed that in the twist curve there is relatively little slope at first, but that this slope increases later; and that at the end of the curve there is a slight but sudden and distinct drop. In other words, at first the internode twists only a little as it grows; later it twists faster; and, when the full length has been attained, there is a slight reverse twist. Note in table I that reading no. 8 shows a slight decrease in length. This was frequently observed at or near the end of growth in other experiments. It is probably due, for the most part at least, to a temporary shortening of the silk thread on account of changes in humidity. It may mean also a slight coiling of the internode. As it probably does not mean an actual shortening, this reading is not plotted in the curve. Reading no. 9 is also omitted from the twist curve.

The rigidity curve shows at first only a slight upward slope, but when the internode is approaching its full length there is a sudden and extensive upward turn in the curve. The coefficient of rigidity

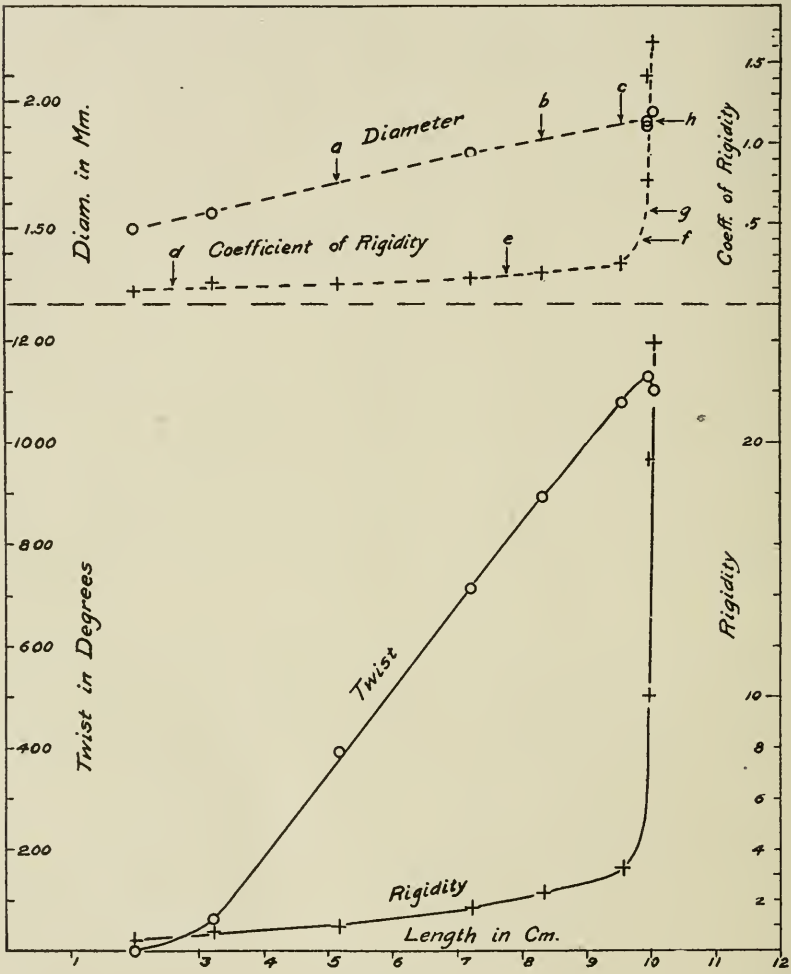


FIG. 5

curve shows the same general properties, but careful study shows that its first slope, that is, up to the point *e*, compared with the initial value of this coefficient, is much less than the ratio between

the corresponding values in the rigidity curve. This demonstrates that increase in diameter accounts to a great extent for increase in rigidity during most of the internode's growth, but does not at all account for the final increase in rigidity.

Many experiments were performed under somewhat different conditions. The results of a few of these are presented graphically

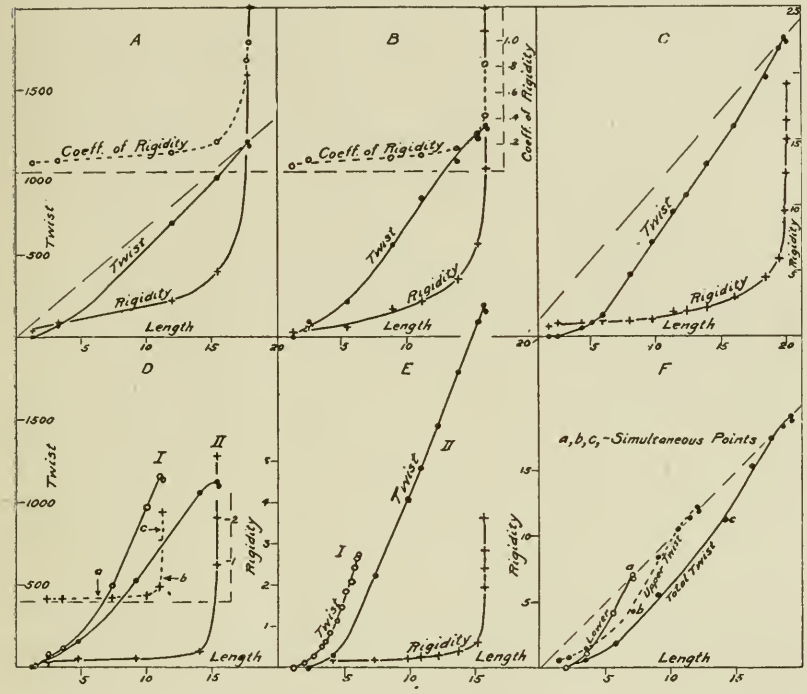


FIG. 6

on a smaller scale in fig. 6, while some of the important data are given for purposes of comparison in table II. The curves of twist, rigidity, and coefficient of rigidity show the same characteristics as those already outlined for fig. 5. It should be mentioned, however, that the final drops in the twist curves in fig. 6 have necessarily been more or less exaggerated. Curves *D*–I and *D*–II are for black bindweed, where the torsion is in the opposite direction from that in the flowering bean, being in the same sense as the thread

of a right-handed screw in the latter, while it is in the same sense as that of a left-handed screw in the former. The rigidity curves for the bindweed are much smaller in dimensions than for the bean. It will also be noticed that differences in diameters account to a great extent for differences in rigidity curves in general.

Curve *E*–I is interesting in that it shows that when the healthy vine is limited in its growth by unfavorable conditions the same rules as to twisting apply. Even in this case there was, as a matter of fact, a slight reverse twist at the end, although the figure does not show it. On the other hand, under distinctly adverse conditions (as when the greenhouse became too hot and too dry), not only was the growth itself stunted, but the torsion was quite irregular. The resulting plant in such a case could hardly be regarded as healthy.

Curves *E*–II are also interesting. Here at the beginning of the experiment the growing tip was purposely removed, but not the leaf at the upper node, which was just above the clamp in the frame. This produced no apparent effect upon the two curves. On the other hand, in some experiments the vine just below the upper node was pinched accidentally, after which that internode died. In this experiment, however, it was observed that the stump above the upper node grew 2 or 3 cm. during the experiment and exhibited apparently a normal amount of twist. In the meantime a new bud appeared at this node.

One more interesting point is shown by curves *F*. Two adjacent internodes were fastened in the apparatus and were measured for length and twist in the usual way, while the behavior of the node between them was studied by means of the mirror clamp (fig. 4). From the resulting data three curves were obtained: one for the part below the node, one for the part above, and one for the total. These curves are plotted together and then a straight line is drawn from the "zero" point to the top of the total curve. The tops of the other two curves fall approximately on this line also. This shows that in a limited portion of the vine, where torsion is taking place freely, the final amount of torsion per unit length is uniformly distributed. If a single internode had been studied in this way, presumably a similar result would have been obtained. This

TABLE II

No.	Date of experiment	Species	Internode	Diam. at end of exp. in min.	Tension net wt. in gm.	Weather conditions	Curves	Remarks
1.....	June 4 to 9, 1918	Flowering bean	7th on branch from first node	1.96	10	Largely cloud	Fig. 5	.....
2*.....	May 31 to June 3, 1918	"	13th	2.25	10	Largely sun	*A* in fig. 6	Final twist = 66° per cm.
3.....	May 26 to 31, 1918	"	11th	2.36	10	Largely cloud	*B* in fig. 6	Final twist = 79° per cm.
4.....	May 10 to 20, 1918	"	5th and 6th	2.1	10	Mostly sun	*C* in fig. 6	Final twist = 90° per cm.
5.....	June 29 to July 4, 1918	Black bindweed	12th	1.0	5	Rain and sun	*D*–I in fig. 6	Twist opposite from bean
6.....	June 26 to 29, 1918	"	17th and 18th	1.3	5	Mostly sun	*D*–II in fig. 6	Twist opposite from bean
7.....	Sept. 21 to 30, 1915	Flowering bean	8th and 9th	About 1.0	13	Sun, rain, cloud	*E*–I in fig. 6	Growth under unfavorable conditions
8.....	June 9 to 17, 1918	"	.....	1.5	10	Mostly sun	*E*–II in fig. 6	Tip removed
9.....	June 12 to 18, 1918	"	11th and 12th on branch at first node	2.1	10	Sun and cloud	*F* in fig. 6	Study of twist in 2 parts

* Nos. 2, 3, and 4 from same plant.

conclusion, however, does not hold for the vine as a whole. This is illustrated by the straight slope lines with curves *A* and *C*, fig. 6, which were obtained from widely separated parts of the same vine. Compare also the final twists per centimeter for nos. 2 and 4 in table II.

Lignification studies

The fact that increase in diameter does not account for the final development of rigidity has already been mentioned. It occurred

TABLE III

LIGNIFICATION TESTS; FLOWERING BEAN

No.	Internode	Length in cm. = L	Period in sec. = T	Diam. in mm. = D	$\frac{L}{T^2D^4}$	Amount of tissue stained with phloroglucin and HCl	Corresponding points on the coefficient of rigidity curve; fig. 5
1..	n*	1.3	1.92	1.5	.07	Only small vessels in vascular bundles	.....
2..	n−1, upper half	1.9	0.93	2.2	.09	Same as in 1, only vessels larger	*d*
3..	n−1, lower half	1.9	0.53	2.5	.17	Vessels larger than in 2; narrow ring of xylem slightly stained	*e*
4..	n−2, middle of upper half	1.8	0.40	2.3	.40	A definite ring of well-stained xylem	*f*
5..	n−2, middle of lower half	1.8	0.40	2.1	.58	Xylem more heavily stained, that is, thicker walled elements	*g*
6..	n−3, upper half	4.0	0.47	2.0	1.14	Xylem still more heavily stained; more contrast between 6 and 4 than between 5 and 4	*h*

* The terminal part; the number of this internode was not counted and so is called *n*.

to the writer that studies in lignification might be useful. For this purpose rigidity and its coefficient were determined on adjacent parts of stems of the flowering bean and of the black bindweed, and free-hand sections from the middle of each specimen were treated with 5 per cent phloroglucin followed by strong HCl.[4] In this way the lignin was stained reddish violet. In tables III and IV the data are given for two such experiments. When the observa-

[4] CHAMBERLAIN, C. J., Methods in plant histology. University of Chicago Press. 1905. p. 62.

tions in table III are correlated with the points *d*, *e*, *f*, *g*, and *h* on the coefficient of rigidity curve in fig. 5, it is seen that the growing internode does not show much rigidity until there is considerable lignified tissue present. For correlation with table IV we choose the rigidity curve of *D*–I, fig. 6, because the final diameter in this

TABLE IV

LIGNIFICATION TESTS; BLACK BINDWEED

No.	Internode	Length in cm. = L	Period in sec. = T	Diam. in mm. = D	$\frac{L}{T^2}$	Amount of tissue stained with phloroglucin and HCl	Corresponding points on rigidity curve; fig. 6, *D*–I
1..	n*	1.1	7.47	0.70	0.02	Only a few small vessels	.....
2..	n−1	1.7	5.03	0.85	0.07	Same as 1, only vessels larger	.....
3..	n−2, upper half	2.0	4.39	0.90	0.10	Vessels still larger and better stained	*a*
4..	n−2, lower half	2.0	1.85	1.00	0.59	Vessels about as in 3; ring of well-stained sclerenchyma	*b*
5..	n−3, upper half	1.7	1.12	1.05	1.35	Sclerenchyma better stained; more of xylem stained	.....
6..	n−3, lower half	1.75	1.00	1.05	1.75	Sclerenchyma still better stained; still more of xylem stained; more contrast between 6 and 4 than between 5 and 4	*c*

* The terminal part; the number of this internode was not determined and so is called *n*.

case is nearly the same as that in table IV. The points *a*, *b*, and *c* again demonstrate that increase in rigidity corresponds with development of lignified tissue.

Antidromous torsion

In the studies on antidromous torsion, a glass rod lubricated with vaseline was attached vertically to the apparatus, so that the frame was allowed to move upward freely but was prevented by this rod from rotating. At the same time the behavior of the midpoint of the part of the vine used was studied by means of the mirror clamp. It was found that this midpoint turned in the same direction as the frame would have done if it had been free to do so. In the case of the bean, for example, under favorable growing conditions the midpoint of an internode was found to twist through

somewhat over 360°. In the meantime a reverse twist was produced in the upper part, which continued to grow in length after the lower part had stopped. In other words, *homodromous torsion* in the lower part overpowered the younger and less rigid upper part and produced *antidromous torsion* in it.

If the frame was released before the internode had stopped growing in length, the upper part recovered to a certain extent from its reverse twist. If this was not done until full growth was attained, it recovered but little or not at all. It does not seem worth while to give results in more detail at present, but it is planned to pursue these points further by modifications of the method.

A few experiments have been made with the first internode of the flowering bean when fastened in this apparatus. It is found to twist in the same direction as other internodes, but only to a limited extent (about 90° or less). Determinations of rigidity gave the same general results as for other internodes. Hop vines were studied to some extent. The indications are that the same general conclusions hold here also, but the experiments were not very complete.

Discussion

The question of reliability of measurements and of results is first to be considered. The errors in measuring increases in length are generally negligible compared with those in other measurements. The first length, however, can be measured only to within about one mm., and this error affects all other lengths, but to a much less extent. Only the first values of rigidity and its coefficient are very much affected by it. As already mentioned, when using the mirror clamp, corresponding lengths can be measured with only fair accuracy. A careful consideration of our readings of the twist shows that these are generally accurate to within 2 or 3°. Disturbing influences, such as air currents and imperfections in apparatus, give at times greater errors than these, and account, in part at least, for slight irregularities in the curves. Determinations of the period of oscillation are generally accurate to about 1 or 2 per cent. Since the square of this value is used in the calculations, this introduces an error of about 2–4 per cent in the values for rigidity. In the last part of each experiment care must be taken to have the

part of the vine within the frame and above the upper clamp as nearly vertical as possible in order to avoid changes in the moment of inertia of the frame, and consequent error in the period of oscillation. Determinations of diameter are least accurate, only to about 3 or 4 per cent, and as the fourth power is used in these calculations, this introduces a further error of about 12–16 per cent in the values for coefficient of rigidity. Moreover, the vines are not perfectly circular in cross-section and are not of uniform texture, as would be the case with a metal wire. For this reason there are objections to the use of the terms rigidity and coefficient of rigidity, in that they do not have as definite values as in physics. When, however, these limitations are borne in mind, and it is remembered that they express relative average values only, it is believed that there should be no confusion. I believe that these errors do not vitiate the conclusions which I have drawn from the curves. It may be well to mention that similar rigidity curves were obtained when the former method of balancing twist in the vine against twist in a fine wire was used.

It may be objected that the material was studied under unnatural conditions, and that stretching introduces an unknown quantity. This is to a great extent true, but it is also true that many other investigations are made under unnatural conditions; for example, histological studies are usually made upon material that has been killed and stained. As a matter of fact, twining plants, when they can get necessary support otherwise, are sometimes observed to twist freely without much coiling or actual twining. This was observed, for example, several times in the greenhouse when a flowering bean grew near a tomato plant and found support among its leaves and branches. It must also be remembered that in ordinary twining the stem is probably subject to more or less tension. What effect, if any, is produced by the amount of tension used, and what effect, if any, the pressure of the clamps has upon the vine can be determined only by further studies.

The first part of the twist curve, which shows only a little slope, may be taken to correspond with that phase of growth which has to do largely with circumnutation, and in which there is but little twist. Torsion of the stem (but not twining proper) is generally

regarded as due to internal forces. It is evident that these forces must reside in the primary tissues, while the final stability of the internode is due to the development of secondary, lignified tissues, corresponding with the last part of the rigidity curve. Apparently lignification stops these internal forces and "sets" the stem with whatever torsion it has acquired. It is very likely that if non-twining stems were studied in a similar way with reference to rigidity similar results would be obtained.

I have not gone into morphological details because I believe that the histology of twining plants with special reference to the points mentioned deserves a more careful study than it has been possible to give it thus far. Further experiments are necessary before any more general conclusions can be drawn.

Summary

The scarlet runner bean and the black bindweed have been studied with respect to torsion in a modified form of auxanometer. It is demonstrated that, as the internode grows in length, at first it twists but little, later it twists much more rapidly, and at the end of its growth in length there is a slight reverse twist. Rigidity, or "resistance to twisting," increases only slowly until near the end of growth in length, when there is a sudden and extensive increase. This final increase in rigidity accompanies the development of secondary, lignified tissues. If we prevent the frame holding the upper part of the internode from rotating, the lower part executes homodromous torsion, and by overpowering the upper part produces antidromous torsion in it. The first internode of the bean twists only a little when held in the apparatus. In a limited portion of the vine when free to twist, the amount of torsion per unit length is about constant, but this is not true of the vine as a whole.

TRAVERSE CITY, MICH.

EARLY DEVELOPMENT OF FLORAL ORGANS AND EMBRYONIC STRUCTURES OF SCROPHULARIA MARYLANDICA

F. M. Schertz

(with plates xxvii–xxix)

The material used in this study was collected at Evanston, Illinois, in the summers of 1909 and 1915, and Bloomington, Indiana, in the summer of 1910. It was killed in medium chromic acetic acid and preserved in 70 per cent alcohol. The paraffin method was used, and difficulty was encountered because of the hardened walls of the seed case. Consequently, the walls of the seed case were dissected away, leaving the ovules attached to the axile placentae, and the difficulty was greatly lessened, although not entirely obviated because of the thick and hardened testa which remained.

Development of floral organs

The order of floral development in *Scrophularia marylandica* L. was found to be calyx, stamens, corolla, and pistil. It seems to be assumed that in the majority of cyclic flowers the parts appear in acropetal succession, namely, sepals, petals, stamens, and carpels. In *Astilbe* Webb has observed that the order of succession of the floral parts is sepals, inner stamens, carpels, outer stamens, and petals. The origin of the petals and the stamens in *S. marylandica* is very similar to that of the flowers of the Primulaceae, in that the primordia of the petals appear after those of the stamens, and each petal apparently comes from the dorsal surface of a young stamen.

Megasporangium

The anatropous ovules arise from central placentae and develop a single integument (fig. 10). Only one layer of cells of the nucellus incloses the mother cell at this stage (fig. 11), but a marked change soon occurs, due to the growth of the integument. This

tegumentary tissue fully surrounds the megaspore mother cell, even as early as the time of the reduction division (fig. 12) of the mother cell. The greatly elongated mother cell forms the four potential megaspores (fig. 13), of which the three potential micropylar megaspores soon degenerate, while the fourth or functioning one forms the embryo sac.

The nuclei of the three degenerating cells (fig. 14) soon disappear, while that of the functional megaspore is clearly visible. In degenerating, the potential megaspore cell next to the functional megaspore disappears first; for a time it forms a sort of cap upon the functional megaspore cell. The three degenerating cells stain very heavily, while the megaspore cell stains lightly. These degenerating cells, which stain deeply with safranin, are last observed as a strip of red above the functional megaspore.

Nucellus

While the megaspore mother cell is being formed (fig. 11), a layer of nucellar tissue envelops it. The rapidly developing integument soon surrounds the nucellus and its mother cell. The cells of the nucellus are long and narrow, and their transverse walls are usually oblique.

A similar tissue arises in many of the other species of the Scrophulariaceae. BALICKA-IWANOWSKI (1) calls it "nucelle," and figures such a tissue in *Uroskineria spectabilis*, *Barisia alpina*, *Pedicularis palustris*, *Klugia notoniana*, *Campanula rotundifolia*, and *Marina longifolia*.

The contents of these cells stain with Delafield's haematoxylin less heavily than the four megaspore cells, and less than the cells immediately surrounding them. As the embryo sac forms, it pushes its way through the micropylar end of the nucellus layer, and when the embryo sac is fully formed the nucellus is found surrounding only the chalazal end of the sac. The nucellus disappears while the endosperm is being formed within the embryo sac. It is still apparent when the first division of the endosperm nucleus takes place, and also until after four cells of the endosperm have been formed. Cells of the nucellus were not observed to be present in later developments of the endosperm (fig. 25).

Tapetal layer

The tapetal layer appears first as a ring or band of cells surrounding the nucellus (fig. 12). The cells of the tapetal layer divide only anticlinally, and later come to form a layer of cells completely surrounding the embryo sac. The cells of this layer lie with their longer axes perpendicular to the embryo sac, while the cells of the nucellus layer within them lie with their longer axes parallel with the embryo sac. This layer of cells is more persistent than the nucellus, and is observed even after most of the endosperm is formed. When the embryo approaches maturity no traces remain of the tapetal layer. These cells possess large nuclei and are well filled with protoplasmic contents. In all of the species of this group as figured by BALICKA-IWANOWSKI, this tapetal layer is shown. The extent to which the tapetal layer incloses the embryo sac varies. In *S. marylandica* the tapetum almost surrounds the embryo sac, or at least to a greater extent than it does in any of the species as figured by BALICKA-IWANOWSKI.

Nutritive tissue

In some species, as *Scrophularia vernalis* and *Scoparia dulcis*, the micropylar end of the tapetal layer is composed of many cells and is called nutritive tissue. In *Digitalis purpurea*, *Linaria Cymbalaria*, and *Torenia Deli* no such tissue is found. At the chalazal end, where the tapetum leaves off, the nutritive tissue completely encircles the lower extremity of the embryo sac in *Scrophularia marylandica* (fig. 22) and in *Linaria Cymbalaria*. In *Scoparia dulcis* no chalazal or micropylar nutritive tissue is observed, and in *Torenia Deli* this tissue is very scanty. The cell walls of the nutritive tissue stain deeply with Delafield's haematoxylin, and the nuclei are less prominent than they are in the surrounding tissues. This seems to indicate that these cells are chemically different from the surrounding tissues.

Embryo sac

The embryo sac develops from the functioning megaspore, and comes to occupy the space formerly occupied by the three potential megaspores. The "nucelle" layer of BALICKA-IWANOWSKI,

judging from the number of cells, does not appear to divide and form new cells after the first division of the megaspore mother cell, but rather, the megaspore by dividing first into two cells (fig. 15), and then into four cells (fig. 16), develops in such a manner as to protrude from the region of the "nucelle." The embryo sac is long and narrow in its chalazal part, and is enlarged in the micropylar end. After the embryo sac reaches the 4-cell stage, as in fig. 16, the nuclei migrate, and the two micropylar nuclei go to the micropylar end of the sac and arrange themselves one on each side of the sac. The nucleus closest to the micropylar end of the embryo sac now divides to form two nuclei, from which the cells of the pear-shaped synergids arise. The other micropylar nucleus divides to form the egg nucleus and the micropylar polar nucleus. The nucleus of the egg had about the same diameter as the synergid nuclei when observed, while the polar nucleus had about twice the diameter of any of the other three micropylar nuclei.

The other two of the four nuclei migrate to the chalazal end of the sac. One of the two chalazal nuclei is found almost at the very tip end of the embryo sac, while the other is located just above it.

It is here that both of these nuclei divide to form the four antipodal nuclei. When the two nuclei divide, their spindles form with the poles toward the ends of the embryo sac rather than crosswise. The three lower nuclei (fig. 18) later become the three degenerating antipodal cells. They degenerate rapidly while the upper nucleus migrates toward the center of the embryo sac, and as it migrates, it greatly enlarges and comes to be as large as the polar nucleus from the micropylar end of the sac. The micropylar polar nucleus and the chalazal polar nucleus now fuse. In the stage before fusion, the nucleoli of both are very prominent (fig. 21), and the nucleus of each is somewhat more than two diameters of the nuclei in the synergids. The large secondary endosperm nucleus now migrates upward toward the central part of the embryo sac, where it is found later, gorged with food.

In *Digitalis purpurea* BALICKA-IWANOWSKI observed remnants of the antipodals, while in *Linaria Cymbalaria* the antipodals are distinct and persist until the complete formation of the chalazal

haustoria. WESTERMEYER, as reported by BALICKA-IWANOWSKI, says that the antipodals of the Scrophulariaceae are particularly difficult to verify.

Embryo

After fertilization the fertilized egg recedes somewhat from the micropylar end of the embryo sac. The first division takes place transversely. It is followed by a longitudinal division in the chalazal cell only. The micropylar cell next divides transversely, giving rise to the first suspensor cells. The two cells of the embryo now divide to form the quadrant stage (fig. 28). The two suspensor cells divide (fig. 34) to form a linear row of four cells, which was the largest number observed. The embryo continues to develop in the normal way to form the mature dicotyledonous type (fig. 35). At no time in its development was the embryo observed to be attached to the micropylar end of the embryo sac by an enlarged basal cell, common in dicotyledons. The embryo of *Plantago maritima* is very similar to the one just described.

Haustoria

In the lower central part of the embryo sac, the secondary endosperm nucleus divides to form endosperm. When the endosperm nucleus has divided to form four cells (fig. 24), the two chalazal cells assume the character of haustoria. The nuclei in them at first (figs. 24, 25) are very prominent, but later seem to degenerate (fig. 29), and when the embryo is fully formed the haustoria themselves entirely disappear.

When the two remaining endosperm cells divide further, they produce four prolongations, which are the micropylar haustoria. These four haustoria are cut off when the endosperm has four cells in cross-section (fig. 25). They may be regarded as absorbing and conducting organs for the transport of food to the rapidly developing endosperm from the surrounding tissues. They are gorged with food matter and react strongly to Delafield's haematoxylin stain. Thickenings of the protoplasm occur in the older haustoria, and may be considered as degenerating nuclei. The nutritive cells of the ovule at the chalazal end of the embryo sac, it may be inferred, are disintegrated by enzymes secreted by the haustoria. Of the

micropylar and the chalazal haustoria, the latter are the larger. As there are no nutritive tissues at the micropylar end of the sac, this accounts for the micropylar haustoria being less developed than those of the chalazal end of the embryo sac. The chalazal haustoria also have a greater region of nutritive tissue from which to absorb. The haustoria attain their greatest development when the endosperm is being formed most rapidly. As the embryo reaches maturity only traces of the haustoria remain.

In the Rhinantheae and other members of the Scrophulariaceae micropylar and chalazal haustoria appear to be quite constant characteristics. The form of the haustoria and the extent to which they are developed vary considerably among the different species, for in *Melampyrum memorosum* they are very arborescent, while in some of the other species only rudimentary haustoria appear. In *Scoparia* no haustoria are noticeable. BALICKA-IWANOWSKI is inclined to think that the micropylar haustoria are not transformed synergids, while SCHLOTTERBECK, to whom reference is made, takes the opposite view. The writer interprets the micropylar and the chalazal haustoria as transformed endosperm cells.

Endosperm

The secondary endosperm nucleus migrates to the lower central part of the embryo sac, and endosperm formation begins with the division of the endosperm nucleus. The secondary endosperm nucleus divides transversely to form the nuclei, and almost immediately a cell wall is formed between them. Each of the two endosperm cells now formed divides longitudinally, and thus four cells are formed. At this stage (fig. 24) the egg and the two synergids were still observed at the micropylar end. The lower two of the above four cells form the haustoria, while the upper two divide transversely and then longitudinally to form eight endosperm cells (fig. 25). At this stage four haustoria are observed at the micropylar end of the embryo sac. They are put out as prolongations of the four micropylar endosperm cells. One cannot regard the micropylar haustoria as transformed synergids. Rapid nuclear division now ensues without reference to these rows of endosperm cells. Endosperm formation takes place before the first division of the egg. As the embryo matures, the surrounding endosperm storage cells

greatly thicken their walls, and their contents consist now of crystalloid protein materials (fig. 36) along with other stored foods.

When the endosperm is formed, it first occupies only the space outlined by the early stage of the embryo sac (fig. 22). As the development of the endosperm proceeds, enzymotic processes set in and the tapetal cells, along with several layers of cells just without, are absorbed and changed into endosperm structure. When the endosperm consists of only two layers of cells throughout the length of the embryo sac (figs. 29, 30, 32), several layers of cells are evident in the surrounding tissues. At a later stage (fig. 33) 8–10 layers of cells were observed in the cross-section of the endosperm, and then not so many layers (3–6) of cells were found in the surrounding tissues. It is evident that the endosperm tissue is being increased at the expense of the surrounding layers of cells. The egg up to this time has not divided. When the egg divides, the endosperm (fig. 26) in cross-section is many layers thick, while the remaining cells of the surrounding tissues have collapsed and become compressed into a thin layer. The cells at the end of the embryo sac are not so greatly changed as are the cells surrounding the sides, and even when the embryo is mature, the cells at the ends of the sac are still noticeable but are slightly compressed. At about the time of the quadrant stage of the embryo (fig. 28), enzymes are secreted and the endosperm tissue surrounding the embryo, extending greatly toward the chalazal end, becomes disintegrated. At this stage the embryo grows rapidly, and soon the cotyledons differentiate. The embryo is now found lying within the endosperm.

The cells of the endosperm are of two types. Those which immediately surround the embryo are long and narrow and mostly devoid of all protein matter, their relation to the embryo evidently leading to a loss of all their cell contents. The cells surrounding these have greatly thickened cell walls and are gorged with protein crystals (fig. 36) and other stored foods. The endosperm is entirely surrounded by a thick hardened coat which stains heavily with Delafield's haematoxylin, and from all appearances serves to protect the seed from loss of moisture. This coat is the testa. Remnants of the single integument persist as an outer covering composed of two or more layers of cells.

One of the rows of cells of the integument has become modified and now appears as a loosely scattered row of cells which connects the outermost layers of cells with the inner thickened hardened layer. Compressed cells, the remnants of the micropylar and the chalazal ends of the embryo sac at the time of fertilization, persist in the seed at each end just outside of the endosperm. The layers of cells covering the seed of various members of the Scrophulariaceae are described by BACHMAN. The number of layers of the testa of the seeds of the 128 species which he describes varies from one to four.

Summary

1. The order of the development of the floral parts of *Scrophularia marylandica* is calyx, stamens, corolla, and pistil. The stamens and the corolla arise from a common outgrowth.

2. The archesporium of the megaspore consists of a single hypodermal cell, which functions as a megaspore mother cell.

3. The megaspore mother cell by two successive divisions gives rise to an axial row of four potential megaspores; the embryo sac arises from the chalazal one, while the other three degenerate.

4. The mature embryo sac contains one egg, two large synergids, an endosperm nucleus, and three antipodal nuclei which soon degenerate.

5. A secondary endosperm nucleus, which grows larger as it migrates toward the egg, was observed. A polar nucleus from the chalazal end was seen to fuse with a polar nucleus from the micropylar end.

6. The first division of the fertilized egg is transverse, and is followed by a longitudinal division of the chalazal nucleus, while the other nucleus fails to divide until later.

7. The nucellus consists of a single layer of cells which surrounds the megaspore.

8. A tapetal layer develops around the embryo sac. It is one cell thick and begins to form at the time the megaspore mother cell divides. At the chalazal end an extensive nutritive layer is formed at the same time.

9. Two well developed haustoria are formed at the chalazal end of the embryo sac. Four less developed ones are formed at the micropylar end of the embryo sac.

10. Only a single thickened integument is found.

11. Endosperm formation takes place before the fertilized egg divides. Endosperm cells separate the fertilized egg from the micropylar end of the embryo sac.

12. The embryo develops a short suspensor which disappears as the embryo matures.

13. The mature seed consists of an embryo surrounded by thickened endosperm cells greatly gorged with crystalline protein and other food matter.

The writer is indebted to Professor C. B. Atwell, of Northwestern University, under whom this work was conducted, for the material upon which the work was done, and for his helpful suggestions and kind advice.

Department of Agriculture
Washington, D.C.

EXPLANATION OF PLATES XXVII–XXIX

Abbreviations used are as follows: *a*, antipodals; *b*, bract; *c*, cotyledon; *co*, corolla; *d*, degenerate nucleus; *e*, embryo; *f*, funiculus; *h*, haustoria; *i*, integument; *j*, endosperm; *k*, micropyle; *l*, egg; *m*, megaspore; *mc*, megaspore mother cell; *n*, nucellus; *o*, ovule; *oc*, ovary cavity; *p*, pistil; *pn*, polar nucleus; *q*, placenta; *r*, rudimentary flower; *s*, stamen; *st*, stigma; *sa*, stem apex; *t*, tapetum; *u*, protein crystals; *v*, testa; *w*, synergid; *x*, calyx; *y*, suspensor; *z*, nutritive tissues.

All of the figures were outlined by means of an Abbé camera lucida on a level with the stage of the microscope. The details were drawn in freehand. The microscope used was a Bausch and Lomb with a triple nosepiece. The following combinations were used: figs. 1–10, no. 1 ocular and 16 mm. objective; figs. 11–19, 21, 27, 31, 34, and 36, no. 8 ocular and 2 mm. objective; fig. 22, no. 8 ocular and 4 mm. objective; figs. 26, 32, 33, and 35, no. 8 ocular and 16 mm. objective; figs. 23, 24, and 25, no. 8 ocular and 3 mm. objective; fig. 20, no. 12.5 ocular and 2 mm. objective. In all of the figures the micropylar end is toward the top of the plate. Figs. 22, 24, 25, and 35 are each reconstructed from several sections.

Fig. 1.—Longitudinal section of stem apex and young bract before any differentiation has taken place.

Fig. 2.—Similar section of flower with bract, showing calyx being differentiated.

Fig. 3.—Similar section of flower showing bract, calyx, and stamens.

Fig. 4.—Similar section of flower, with calyx and stamens; petals and pistil appearing.

FIGS. 5, 6.—Parts in fig. 4 at an older stage.

FIG. 7.—Ovary cavity and stigma being differentiated.

FIGS. 8, 9.—Showing common origin of stamens and petals, differentiation of ovary, and relation of floral parts.

FIG. 10.—Section showing pistil and ovules on axile placenta.

FIG. 11.—Young ovule showing archesporial mother cell and nucellus, also integument.

FIG. 12.—Young ovule showing megaspore mother cell.

FIG. 13.—Axial row of 4 megaspores; nucellus, tapetum, and integument also shown.

FIG. 14.—Axial row of 4 megaspores; 3 micropylar ones degenerating.

FIG. 15.—Two-nucleate embryo sac.

FIG. 16.—Four-nucleate embryo sac.

FIG. 17.—Micropylar end of mature embryo sac.

FIG. 18.—Chalazal end of mature embryo sac.

FIG. 19.—Embryo sac showing antipodal polar nucleus, two synergids, egg, and micropylar polar nucleus.

FIG. 20.—Two synergids and egg.

FIG. 21.—Fusion of 2 polar nuclei.

FIG. 22.—Ovule just before fertilization and its relation to nucellus, tapetum, and nutritive tissue; funiculus also indicated.

FIG. 23.—First division of endosperm nucleus forming 2 cells.

FIG. 24.—Four cells of endosperm; 2 chalazal ones later form haustoria.

FIG. 25.—Two chalazal haustoria; the 4 micropylar haustoria and the 8 endosperm cells separating the haustoria.

FIG. 26.—The 3-celled embryo in surrounding endosperm cells in longitudinal section.

FIG. 27.—The 3-celled embryo; single cell later forms suspensor, while the 2 cells later form embryo.

FIG. 28.—Cells dividing to form quadrant stage; 2 suspensor cells shown.

FIG. 29.—Relation of chalazal haustoria to nutritive tissue; tapetum and endosperm cells.

FIG. 30.—Relation of micropylar haustoria to tapetum.

FIG. 31.—Cross-section through central region of embryo sac showing endosperm division into 2 rows of cells throughout the sac.

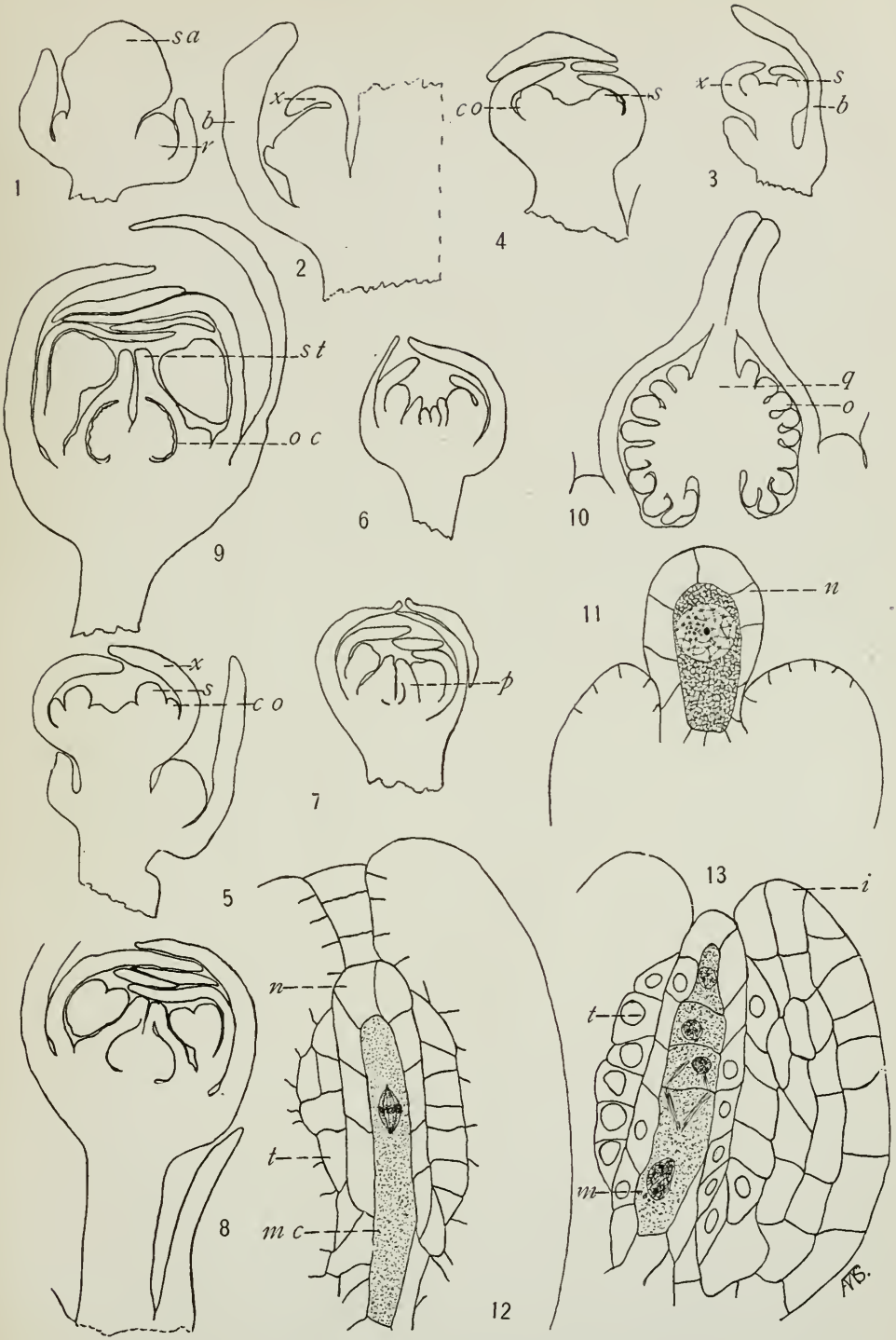
FIG. 32.—Outline drawing showing micropyle, funiculus, and relation of the 2 rows of endosperm to rest of ovule.

FIG. 33.—Later stage showing increase of endosperm in proportion to rest of ovule.

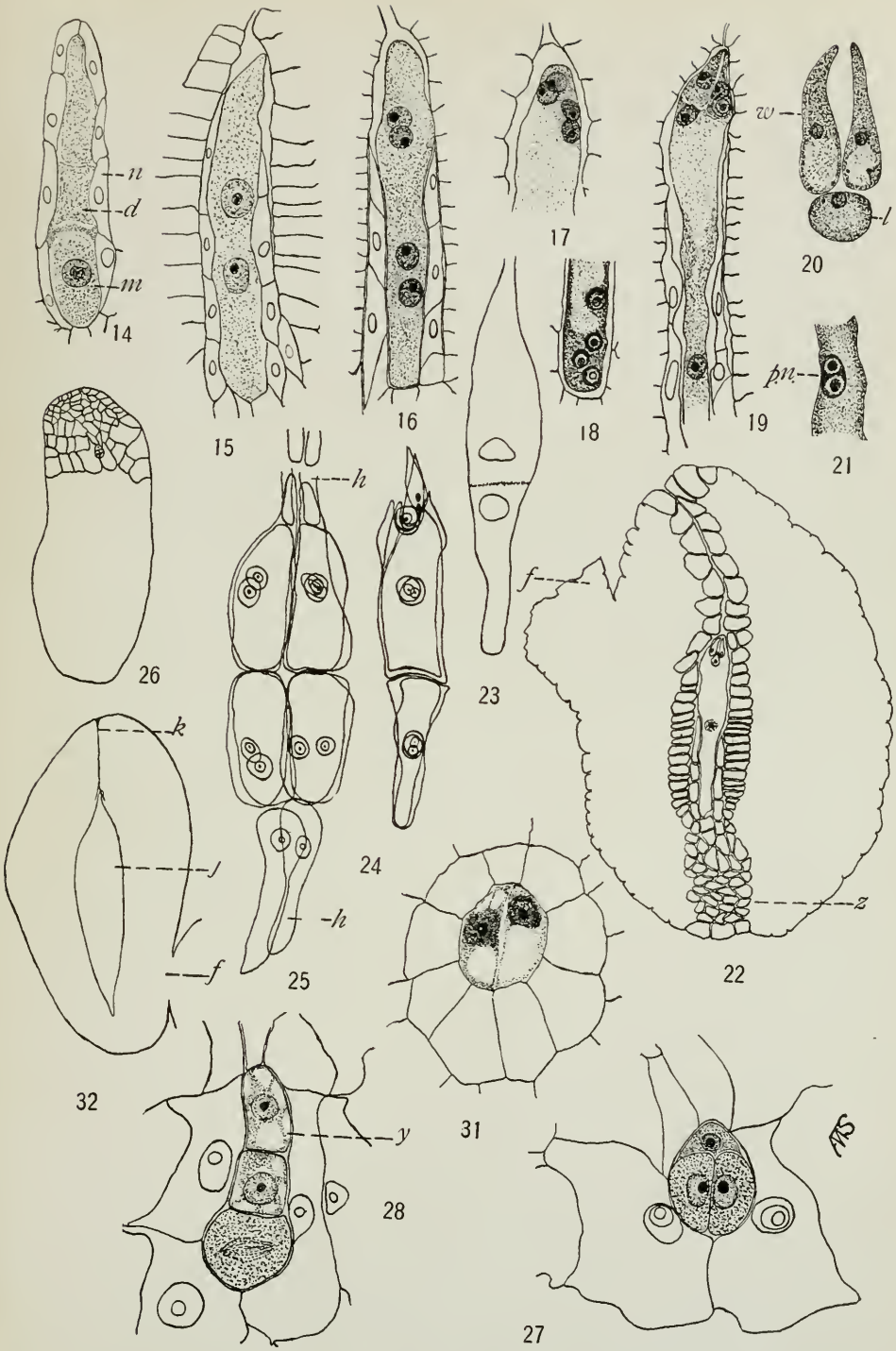
FIG. 34.—Young embryo showing the 4 cells in suspensor.

FIG. 35.—Cross-section through mature seed showing embryo, thickened endosperm cells filled with protein, and remains of integument which now functions as the testa.

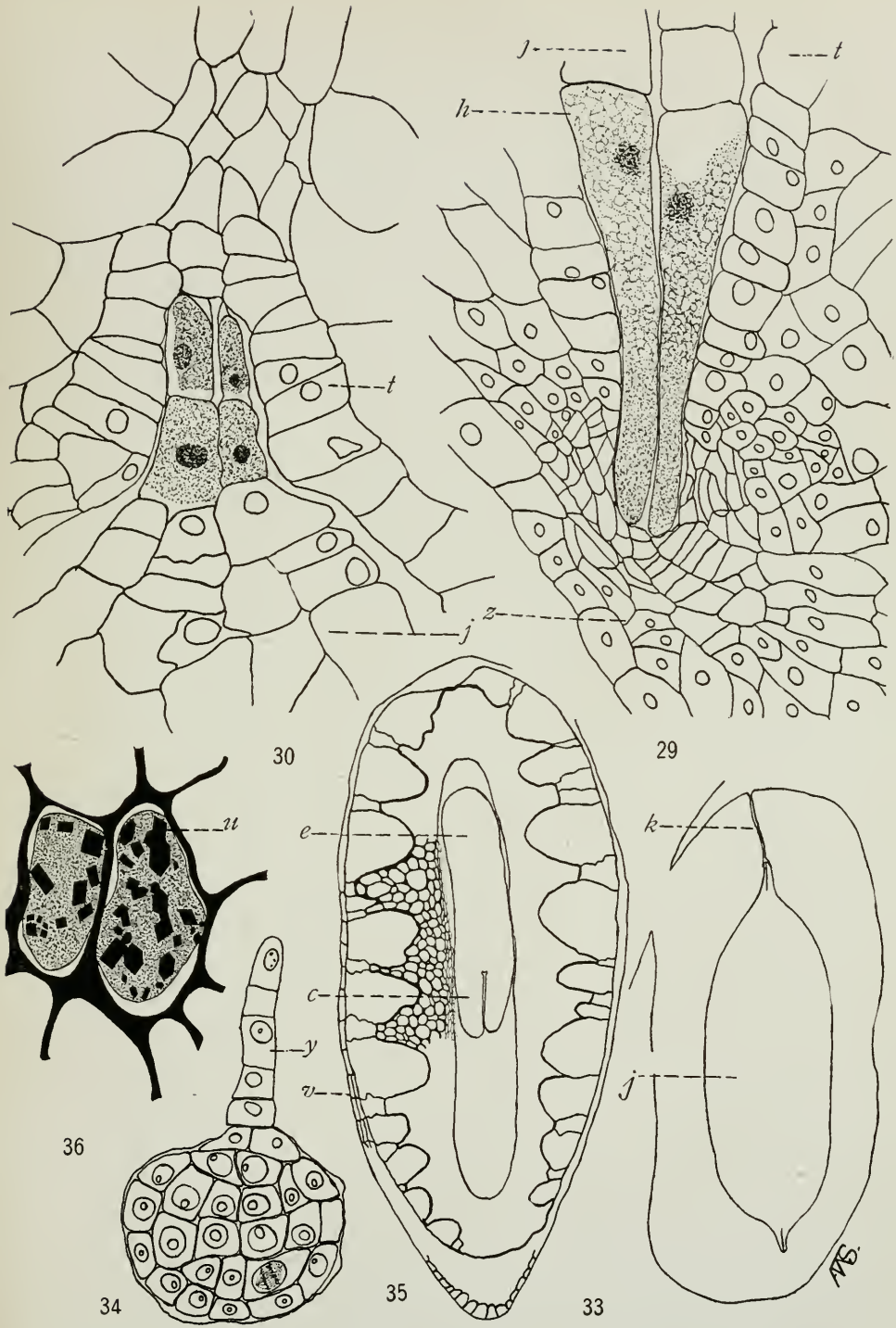
FIG. 36.—Enlarged drawing of thick-walled protein-filled endosperm cells.



SCHERTZ on SCROPHULARIA



SCHERTZ on SCROPHULARIA



SCHERTZ on SCROPHULARIA

COMPANION CELLS IN BAST OF GNETUM AND ANGIOSPERMS

W. P. Thompson

(WITH SEVEN FIGURES)

The presence of companion cells in the bast of angiosperms is one of the constant anatomical features which distinguish that great group of plants from the gymnosperms. On account of the technical difficulties in the study of bast tissue in general, little emphasis has been attached to this distinction, although it is really quite as valuable as the familiar one based on the presence of vessels in angiospermic wood. The elements in question are designated companion cells, because almost invariably one of them is associated with each sieve tube. They are small, vertically elongated, parenchymatous cells which have special characteristics that will be described later, and which have no counterpart in the bast of gymnosperms.

In a study of the anatomy of the Gnetales (3, 5), it became clear that the same elements or ones remarkably similar are to be found in the bast of *Gnetum*. Another clear-cut characteristic is therefore added to the long list of features in which *Gnetum* departs from gymnospermic structure and resembles angiosperms. It has been shown (5), however, that one of the most striking of these resemblances, the possession of vessels, is not the result of a genetic connection between *Gnetum* and angiosperms, because the vessels have been evolved in entirely different ways in the two groups. It becomes necessary, therefore, to examine and compare the angiospermic and Gnetalean companion cells both as to structure and as to development.

Companion cells of angiosperms

Angiospermic bast as found in *Aristolochia macrophylla* (Lam.) is represented in fig. 1. Two chief kinds of elements are visible: the clear, irregularly shaped sieve tubes, and the richly protoplasmic parenchymatous cells (in most sections of bast there are

in addition thick-walled fibers constituting the so-called hard bast). The parenchymatous elements are in turn plainly of two kinds: large cells containing starch grains, which are represented in black as if stained with iodine, and much smaller, starchless elements. The latter are the companion cells, whereas the former are ordinary storage parenchyma. A further difference between the two kinds appears in longitudinal sections; the companion cells are highly elongated vertically, while the ordinary storage parenchyma cells are rectangular. It should be noted that a

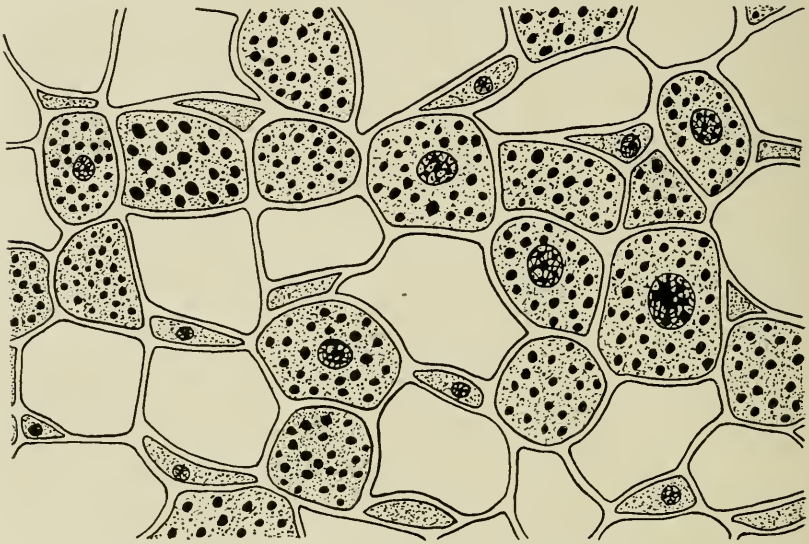


FIG. 1.—Mature bast of *Aristolochia macrophylla* (Lam.); ×1000

companion cell is associated with each sieve tube and is frequently fitted snugly into one corner of the tube.

The bast of *Aristolochia* was chosen for illustration because its elements exhibit a more orderly arrangement than is usual in angiosperms. In many species the rows of phloem cells formed at the cambium very quickly become so distorted that the orderly sequence is lost, and the cells of the mature bast then appear to be haphazardly placed. In *Aristolochia*, however, the rows of cells are to a certain extent preserved in the mature bast, as may be seen in fig. 1. When this is the case, there is a significant

indication of relationship between sieve tube and companion cell. The relative positions of these two elements is such as to indicate that they are formed from two successive cells in a row, and that they are products of the same mother cambial cell. This relationship was mentioned long ago by DeBary (1): "Both from their arrangement in cross-section and on tracing them in the longitudinal direction, it often has the appearance as if the cambiform cells arose with the elements of the sieve tubes from one mother

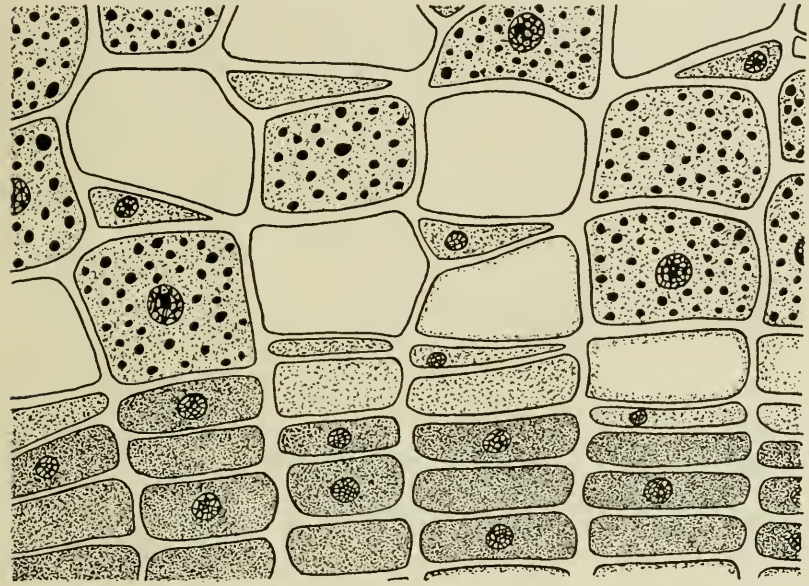


Fig. 2.—Cambium and young bast of *Aristolochia macrophylla* (Lam.) showing each sieve tube and its companion cell formed from contiguous cells in same row; ×1000.

cell, the latter dividing longitudinally into a daughter cell which becomes the sieve tube element, and another which becomes a cambiform cell without further division or is divided by cross walls into several of them." Strasburger (2) later made the definite statement that companion cells and sieve tubes are sister cells.

This statement is of course to be confirmed or disproved by an examination of the method in which the bast develops in the cambial region. The process as it occurs in *Aristolochia* is illustrated in fig. 2. As one traces the rows of cambial cells in the

lower part of the figure into the bast of the upper part, it becomes perfectly clear that each sieve tube and its companion cell are derived from contiguous cells in the same row, and that as the bast matures the companion cell tends to become shifted to one corner of the sieve tube. I have not been able to show that sieve tube and companion cell are products of one division. In fact, judging from what we know of cambial activity, it seems more probable that they are products of two successive divisions, in

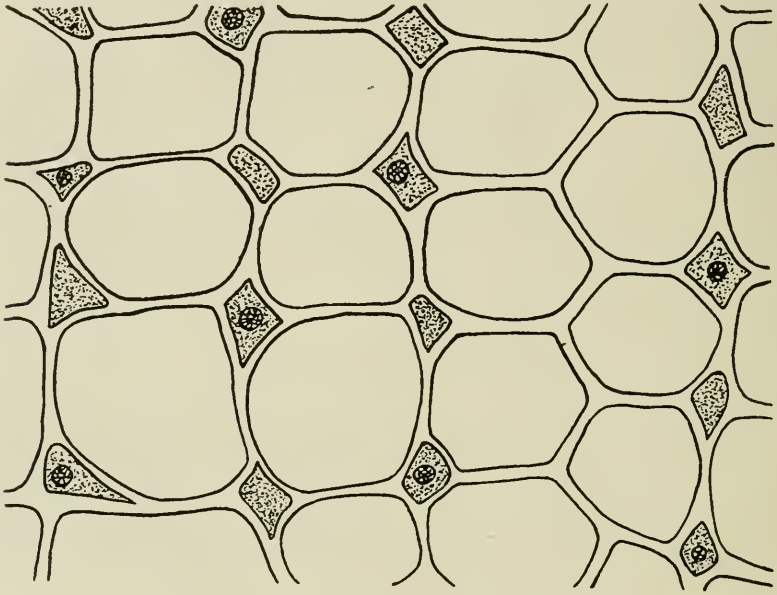


FIG. 3.—Mature bast of *Gnetum latifolium* showing sieve tubes and companion cells; ×1000.

each of which one daughter (the innermost) remains cambial. In other words, they are probably not sisters but aunt and niece. However that may be, for the purposes of the present discussion the important conclusion is that they are successive members of a single row of cambial products.

Companion cells of Gnetum

The mature bast of several species of *Gnetum* (fig. 3) consists of only two kinds of elements, large clear sieve tubes and small parenchymatous cells. The latter evidently correspond to the

companion cells of angiosperms, as is shown by their small size, their association with sieve tubes, their location in the corners of the sieve tubes, their lack of starch, and their great elongation in the vertical direction. Storage parenchyma of the ordinary type (not rays) as found in angiosperms is absent. Sieve tubes and companion cells are arranged with great regularity, the former in very uniform rows and the latter in the angles between the tubes. Usually there is an interrupted row of companion cells for every row of sieve tubes, but occasionally the former are lacking, as may be seen toward the right of fig. 3.[1]

The development of the bast at the cambial region is illustrated in fig. 4. It will be seen that the companion cells are formed in radial rows which are continuous through the cambium, and that in these rows sieve tubes are not formed. There is a tangential alternation of cambial rows which form sieve tubes with cambial rows which form companion cells. Both are never formed in the same row. As the bast matures and the sieve tubes expand, the rows of companion cells become interrupted and the individual cells pushed to the corners of the sieve tubes.

This process is evidently quite different from that which occurs in angiosperms, because in the latter sieve tube and companion cell are invariably successive cells in the same row. There is no separate cambial mother cell for the companion cells. Thus the similar mature condition is brought about in quite different ways in the two groups.

A study of the wood adjacent to the cambium, also illustrated in fig. 4, reveals an unexpected relationship between the companion cells and the parenchyma of the wood. The same cambial rows which form companion cells outwardly also form rows of wood parenchyma cells inwardly. The latter with their protoplasm are easily distinguished in the figure from the empty, thick-walled wood fibers which are formed in rows by the same cambial cells which form sieve tubes. The rows of wood parenchyma formed in this way are readily mistaken for uniseriate rays, but longitudinal

[1] It may be remarked that the bast of *Gnetum* is extremely favorable material, both for original study and for class use. The sieve areas are remarkably abundant and of great size, and the individual pores are very large and clear. Moreover, the whole tissue and the connected cambium are not easily crushed in sectioning.

sections show that they are typical, vertically elongated wood parenchyma. The rays of *Gnetum* are multiseriate or broad.

The appearance of this parenchyma in the mature wood is shown in fig. 5. At the end of the season's growth, where the fibers are small and vessels lacking, the wood parenchyma forms continuous rows (lower part of figure), but with the increase in size of the fibers and the introduction of vessels at the beginning of the next season's growth, the parenchymatous cells become

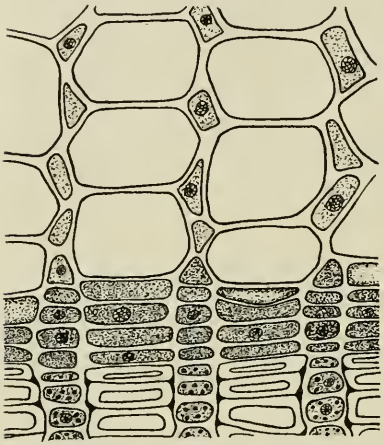


FIG. 4

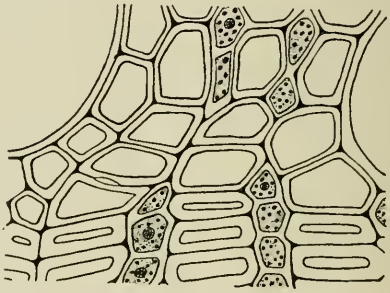


FIG. 5

FIGS. 4–5.—Fig. 4, wood, cambium, and young bast of *Gnetum latifolium* showing sieve tubes and companion cells formed from different rows of cambial cells; also wood parenchyma formed inwardly from those cambial cells which form companion cells; ×1000; fig. 5, wood of *Gnetum latifolium* showing radial rows of wood parenchyma; ×1000.

separated just as do the companion cells of the bast. Often where the vessels are large the rows of elements of all sorts become so distorted that they can no longer be traced. The elements then appear to be haphazardly arranged.

In certain regions of some species of *Gnetum* particularly interesting and primitive conditions of the companion cells are found. In the root of *G. scandens*, for example, the companion cells do not become separated and relegated to the corners of the sieve tubes, but form continuous rows, even in old bast. This condi-

tion of affairs is illustrated in fig. 6. At the top of the figure, although the bast is old and beginning to collapse, the companion cells are still in continuous rows. This condition is frequently found in roots, young stems, and reproductive axes. It evidently represents a retention of a primitive condition.

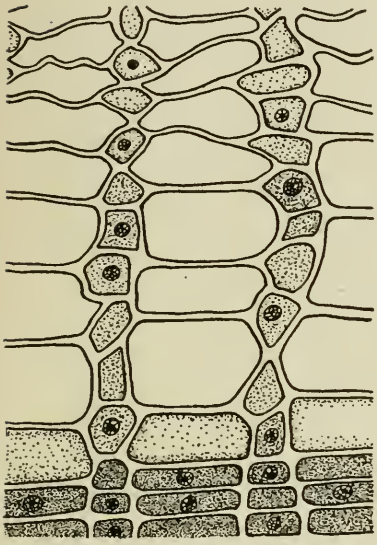


FIG. 6

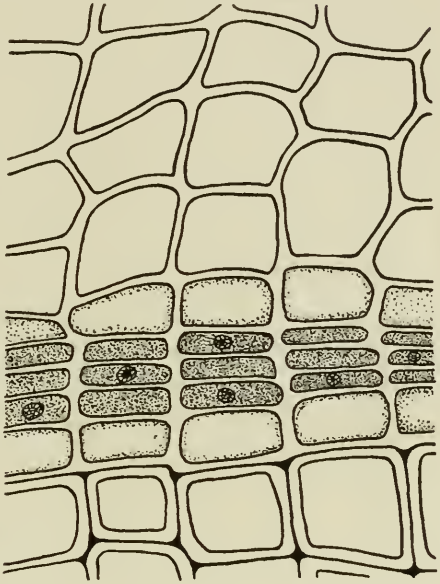


FIG. 7

FIGS. 6–7.—Fig. 6, cambium and bast of root of *Gnetum scandens* showing uninterrupted rows of companion cells; ×1000; fig. 7, wood, cambium, and bast of young stem of *Gnetum moluccense* showing absence of companion cells and wood parenchyma; ×1000.

Again, in certain regions of some species companion cells are entirely absent, the bast then consisting entirely of sieve tubes and rays. This is illustrated in fig. 7, from the young stem of *G. moluccense*. When this is the case, wood parenchyma is also lacking, as might be expected from the relationship which has been shown to exist between companion cells and wood parenchyma. The absence of companion cells has been noted in seedlings of several species and in reproductive axes, as well as in roots and young stems.

Discussion

The presence in the bast of *Gnetum* of companion cells which are in most respects of the angiospermic type is at first sight another striking indication of real relationship between *Gnetum* and angiosperms. They are to be compared with the vessels of the wood, broad rays, general habit, style (4), absence of archegonia, free-nucleate embryo sac, endosperm formation (4), and other reproductive characters. In regard to all of these points *Gnetum* is angiospermic.

The study of the development of the companion cells, however, shows that the resemblance does not necessarily indicate genetic relationship. Whereas the companion cells of angiosperms are formed from the same row of cambial cells as are the sieve tubes, and each one is contiguous to a sieve tube in such a row, those of *Gnetum* are formed in rows quite separate from the sieve tubes and are the products of different cambial cells. Of course, it is possible that the companion cells of *Gnetum* are really genetically related to those of angiosperms, and that, after originating in *Gnetum* in the method described, their formation has later been taken over in angiosperms by the same cambial cells that form sieve tubes. On the other hand, it seems more logical to conclude that we are dealing with a case of parallel evolution, just as I have shown to be true with regard to the vessels of the wood (5). The vessel of *Gnetum* with a single large perforation in the end wall is almost identical with that of many angiosperms. Nevertheless, it has been evolved in an entirely different way. The perforation of the angiospermic porous vessel has resulted from the breaking down and disappearance of scalariform bars, whereas that of *Gnetum* has resulted from the enlargement of typical, circular, haphazardly arranged, bordered pits accompanied by the disappearance of the middle lamellae, the enlargement proceeding until the intervening portions of the vessel wall have vanished. In the case of the companion cell we seem to have a close parallel to that of the vessel. While the completed structure is similar to that of angiosperms, the course of its development is quite different.

If two such striking points of resemblance as vessels in the wood and companion cells in the bast are really results of inde-

pendent evolution in *Gnetum* and angiosperms, the inference is natural that other resemblances may be in the same category.

Summary

1. Companion cells resembling those of angiosperms in size, in their association with sieve tubes, in their usual location in the angles of the sieve tubes, and in their vertical elongation, are present in the bast of some species of *Gnetum*.

2. The development of these companion cells, however, is quite different from that found in angiosperms. Whereas, in the latter, each sieve tube and its companion cell are derived from two successive cells in a single row of cambial products, in *Gnetum* sieve tubes and companion cells are produced from different rows of cambial cells.

3. Although the completed forms of companion cell in the two groups are similar, they have probably been independently evolved.

4. Primitive conditions in which companion cells are lacking, or in which continuous rows of companion cells are present, are found in certain regions of some species.

5. The parenchyma of the wood is formed by those cambial cells which form companion cells; the distribution of the wood parenchyma is consequently in radial bands, which frequently become interrupted by the expansion of vessels and fibers.

UNIVERSITY OF SASKATCHEWAN
SASKATOON, SASK.

LITERATURE CITED

1. DEBARY, A., Comparative anatomy of Phanerogams and Ferns. Oxford University Press. 1884.
2. STRASBURGER, NOLL, SCHENK, and KARSTEN, Textbook of Botany. Macmillan. 1912.
3. THOMPSON, W. P., The anatomy and relationships of the Gnetales. 1. The genus *Ephedra*. Ann. Botany **27**:1077–1102. 1912.
4. ———, The morphology and affinities of *Gnetum*. Amer. Jour. Bot. 4:135–184. 1916.
5. ———. Independent evolution of vessels in Gnetales and Angiosperms. BOT. GAZ. 65:83–90. 1918.

SECRETION OF AMYLASE BY PLANT ROOTS[1]

L. KNUDSON AND R. S. SMITH

(WITH TWO FIGURES)

The fact that green plants are able to absorb certain organic substances by means of the roots, and to utilize these substances, suggests the question whether the roots of plants secrete enzymes in a manner comparable to various fungi, digesting in the culture medium, etc., the various organic substances that might be supplied. Various investigators have incidentally touched the subject, but the evidence obtained is conflicting and not at all conclusive.

LAURENT (3) reported the inversion of saccharose when this sugar was present in the culture media, and he ascribed this to the enzyme invertase secreted by the roots of corn or of peas. Starch was likewise transformed, but LAURENT ascribed this transformation to diastase secreted by the seed. MAZÈ (4) reported inversion of saccharose, but in 1911 (5) he stated that there was no enzyme secretion by the roots, and that starch was absorbed directly. WOHLLEBE (8), investigating the secretion of amylase by roots, came to the conclusion that there was a very weak secretion of amylase by the root hairs, and in some cases secretion of amylase was effected by the disconnected root-cap cells. The senior writer of this paper suggested in a previous publication (2) that invertase is secreted by the roots.

In view of the indefiniteness of information on the subject, it seemed advisable to investigate the problem. The first experiments were made on the secretion of amylase, and the results obtained constitute the basis for this paper.

Pfeffer's was the nutrient solution employed. It was made up according to the following formula: $Ca(NO_3)_2$ 4 gm., KNO_3 1 gm., K_2HPO_4 1 gm., $MgSO_4$ 1 gm., KCl 0.5 gm., $FeCl_3$ 100 mg., distilled water 6 l. Merck's soluble starch was used throughout the experiments.

[1] Contribution from the Laboratory of Plant Physiology, Cornell University.

The plants were grown under sterile conditions.[2] The seeds were sterilized by the calcium hypochlorite method (7). After the seeds were sterilized, they were planted in test-tubes on sterilized

FIG. 1

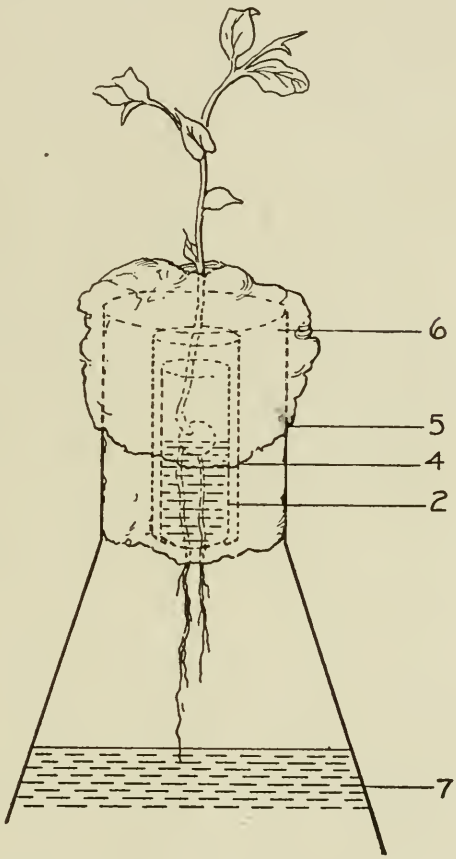


FIG. 2

FIGS. 1, 2.—*1*, sterile culture tube; *2*, germination tube; *3*, 1 per cent agar; *4*, outer tube; *5*, Erlenmeyer flask; *6*, sterile cotton; *7*, nutrient solution.

agar, as shown in fig. 1. When the seedlings were sufficiently developed, they were transferred to the culture vessels. The culture vessels used were Erlenmeyer flasks of 1 or 2 l. capacity.

[2] The writers are indebted to Dr. J. K. WILSON for the method of growing plants under sterile conditions.

The flasks were stoppered with cotton plugs, each provided with a glass tube passing through the center, this tube also being plugged with cotton. The culture flasks with solutions were sterilized in the autoclave for 30 minutes at 15 pounds pressure.

When the seedlings were of adequate size, they were transferred to the culture vessel. Transfer was made when the roots reached the bottom of the tube and were curled about, and the tops had attained a height of about 5 cm. By use of a heavy platinum needle, the tube, together with the inner core of agar and the seedling, was withdrawn from the test-tube and transferred to tube 4 of the culture vessel (fig. 2). The tube 4 was slightly drawn at the base so as to prevent tube 2 from passing through into the culture solution. Sterilized cotton was then packed about the seedling in tubes 2 and 4. The cultures when set up appeared as shown in fig. 2. After being kept for a few days in the laboratory, the cultures were transferred to the greenhouse.

The particular advantage of this form of culture, from the standpoint of studying the secretion of enzymes by the roots, is that the seeds are kept entirely out of contact with the culture solution, and any enzymes derived from the seeds are held in the agar, which with the passing of time loses its water and hardens to a flaky mass.

In spite of all the precautions taken, cultures occasionally became contaminated. All such cultures were rejected. At the conclusion of the experiment, the culture solutions were brought to the original volume and analyzed. In the first cultures, tests were made for contaminating organisms, but these and other similar experiments indicated that if the culture solutions were clear at the conclusion of the experiment there was no contamination. Consequently, in the later experiments, no platings were made of the culture solutions. Data were collected also on the weights of roots and tops. Detailed methods of procedure are described under the different experiments.

For the first experiment two cultures were set up, following the method described, using 2 l. flasks, in each of which was placed 1800 cc. of the culture solution containing 0.25 per cent of soluble starch. A variety of corn known as Leaming was employed. The

cultures were grown in a greenhouse, under favorable conditions, from November 14, 1916, to December 5, 1917. One of the cultures became contaminated, so that data were obtained from only one culture. Reducing sugar was determined by KENDALL'S method (1).

The dry weights of roots and tops were respectively 172 and 430 mg.; the total weight was 602 mg.; and the increase in weight over the original weight of the seed was 262 mg. Determinations made of the reducing sugar in 100 cc. lots of both the culture solution and the control solution gave 22.18 mg. of copper for the former, and 11.25 mg. for the latter; or an increase of 10.93 mg. of copper for a 100 cc. solution (about 6 mg. of maltose). A sample of the culture solution and one of the control, with 2 per cent of toluene added to each, were incubated for one week at 30° C., and showed no increase in reducing sugar.

In this preliminary experiment there was noted a slight increase in reducing sugar, but the increase was so small as to be without significance. Furthermore, the fact that there was no increase of reducing sugar on incubation leads to the conclusion that the enzyme amylase was not secreted into the culture solution.

The conditions of the second series of experiments were the same as for the preceding, except that liter flasks were employed as culture vessels, and 1100 cc. of the culture solution was used. A variety of white dent corn known as Boone County White was used. The cultures were grown for a period of 51 days.

At the conclusion of the experiment, the culture solutions were brought to their original volume and samples were kept for analysis. To the sample solutions was added 2 per cent of toluene, and two weeks elapsed before the solutions were analyzed for reducing sugars. Analyses were made by the Munson Walker method. Data are given in table I. The data indicate that in the culture solutions there is a slight increase in reducing sugars, but not sufficient to warrant the conclusion that there is any amylase secretion. The objection might be raised that in these cultures there can be no accumulation of reducing sugars, because they are utilized as fast as produced, which is possible; but the fact that the increase is so slight, even after two weeks of incubation, supports the theory that no amylase was present.

In the third experiment the procedure was similar to that in the preceding experiments. A white dent corn was used, and also Canada field pea. In addition to using Pfeffer's solution plus soluble starch, a number of cultures were made in which Pfeffer's solution alone was employed, to see whether any reducing sugars were secreted. Two liter flasks were used. The concentration of starch was approximately 0.35 per cent. The duration of the

TABLE I

Culture solution	Dry weight (in gm.)				Reducing sugar as CuO (in mg.)	
	Roots	Tops	Total	Gain	In 100 cc. sample	Increase per 100 cc.
Pfeffer's solution	0.6	1.05	1.65	1.3	None	None
Pfeffer's solution +0.25 per cent starch	0.5	2.1	2.6	2.3	14.5	6.4
Pfeffer's solution +0.25 per cent starch	0.5	1.7	2.2	1.9	10.0	1.9
Pfeffer's solution +0.25 per cent starch	0.7	2.15	2.85	2.55	12.9	4.8
Pfeffer's solution +0.25 per cent starch; no plant	.....	.....	.....	.....	8.1	.....

experiment was 47 days. At the conclusion of the experiment, the culture solution and the controls were made up to their original volumes, and 20 cc. portions were taken and were analyzed by Shaffer's method (6) for reducing sugars. Sample lots of each were also incubated with 2 per cent of toluene at 32° C. for 10 days, and the reducing sugars again determined. The data are given in table II.

There is a very slight increase in reducing sugars in some of the culture solutions over that in the control, but not enough to be of any significance. Furthermore, after 10 days' incubation there was no increase in the amount of reducing sugars.

Finally, to prove that the soluble starch is not utilized directly or indirectly to any appreciable extent, the following procedure was undertaken. Sample lots of the control and culture solutions were hydrolyzed and reducing sugars were determined. It was found that the control and culture solutions showed the same amount of

reducing sugar, which was equivalent to 73 mg. of glucose per 20 cc. of the culture solution.

In conclusion, therefore, it may be stated that neither *Zea mays* L. nor *Pisum arvense* L. is capable of utilizing soluble starch

TABLE II

Plant	Culture no.	Culture solution	Dry weight (in gm.)			Reducing sugars (in mg.)	
			Tops	Roots	Total	At termination of experiment	After 10 days' incubation
Corn	1	Pfeffer's solution alone	1.95	1.00	2.95	Trace	.....
	2		1.80	0.75	2.55	"	.....
	3		1.70	0.95	2.65	"	"
Pea	1		0.68	0.26	0.94	"	.....
	2		0.55	0.07	0.62	"	.....
	3		0.64	0.18	0.82	"	.....
Corn	1	Pfeffer's solution +0.35 per cent of soluble starch	0.7	0.4	1.10	3.4	4.0
	2		1.4	0.6	2.00	6.5	6.0
	3		1.5	0.7	2.20	3.7	4.0
	4		1.1	0.5	1.60	5.3	5.0
	5		1.4	0.6	2.00	4.4	4.0
Pea	1	Pfeffer's solution +0.35 per cent of soluble starch	0.5	0.2	0.70	3.7	4.0
	2		0.5	0.2	0.60	6.5	6.0
Control	1	No plant	.....	.....	.....	1.8	.....
	2		.....	.....	.....	3.7	.....

directly or indirectly, nor is there any appreciable secretion of amylase by the roots of these plants, at least under conditions such as were maintained for these experiments.

Cornell University
Ithaca, N.Y.

LITERATURE CITED

1. Kendall, E. C., A new method for the determination of reducing sugars. Jour. Amer. Chem. Soc. 34:317–341. 1911.
2. Knudson, Lewis, Influence of certain carbohydrates on green plants. Cornell Univ. Agric. Exp. Sta. Memoir 9:9–75. 1916.
3. Laurent, J., Recherches sur la nutrition carbonée des plantes vertes a l'àide de matières organiques. Rev. Gén. Bot. 16:14–48, 68–80, 96–119, 120–128, 155–166, 188–202, 231–241. 1904.

4. MAZÈ, P., L'assimilation des hydrates de carbone et l'élaboration de l'azote organique dans les végétaux supérieurs. Acad. Sci. Paris. Compt. Rend. **128**:185–187. 1899.
5. ———, Influence, sur le développement de la plante, des substances minérales qui s'accumulent dans ses organes comme résidus d'assimilation. Absorption des matières organiques colloidales par les racines. Acad. Sci. Paris. Compt. Rend. **152**:783–785. 1911.
6. SHAFFER, P. A., On the determination of sugar in blood. Jour. Biol. Chem. **19**:285–295. 1914.
7. WILSON, J. K., Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**:420–427. 1915.
8. WOHLLEBE, H., Untersuchungen über die Ausscheidung von diastastischen und proteolytischen Enzymen bei Samen und Wurzeln. Inaug. Diss., Leipzig. p. 1–35. 1911.

RAY TRACHEID STRUCTURE IN SECOND GROWTH SEQUOIA WASHINGTONIANA

H. C. Belyea

(WITH FIVE FIGURES)

Ray tracheids are essentially a structural element in all woods of the Coniferae, and, as has been pointed out by several writers, the value of their presence or absence in taxonomy is without question. It has also been stated by several writers on this subject that as a wood structure they reach their highest development in their normal occurrence in the Pineae of the Abietineae, particularly in *Larix*, *Picea*, *Pseudotsuga*, and most notably in *Pinus*, attaining their greatest complication in the dentations and reticulations of the marginal ray tracheary cells of the hard pines. In the other two members of the Pineae, ray tracheids are normally found in *Tsuga*, but not in *Abies*. Although DeBary (1) and Penhallow (6) both described them as characteristic of *A. balsamea*, this conclusion does not seem to be borne out by the work of Thompson (7) and Miss Holden (4), who both state that ray tracheids are not to be found in this species. Thompson (7), however, reports them as occurring traumatically in *A. amabilis* and *A. concolor*.

In the Taxodineae, while they are entirely absent from *Taxodium*, they are notably present in *Sequoia*, and have been described by Gothan (3) for *S. washingtoniana*, and for *S. sempervirens* by Miss Gordon (2) and Jones (5).

In the Cupressineae they are found in all members of *Chamaecyparis*, more or less abundantly in *C. nootkatensis*, sparsely in *C. Lawsoniana*, and, according to Thompson (7), only under traumatic stimulus in *C. thyoides* and *C. plumosa*.

In the closely allied genus *Cupressus* they are much more abundant, frequently occurring as an entire ray one to three cells high. In *Thuya* they are also quite common, and in this genus are invariably marginal, with small bordered pits on their tangential walls, and slightly larger ones on their lateral end walls. In *Juniperus*

they are present very sparsely, but are readily recalled under traumatic stimulus. In this genus, according to Miss HOLDEN, ray tracheids usually occur as very irregularly walled cells, thickly pitted on the tangential walls, usually constituting a ray one cell high. According to PENHALLOW there are no ray tracheids in *Libocedrus*, but Miss HOLDEN reports them sparsely located under traumatic stimulus in wounded material from *L. decurrens*.

It is to be noted that while ray tracheid structure is an essential feature of the Coniferales, it is only constantly and normally present in the older genera. In the younger genera this structure may or may not be present, yet is invariably recalled under traumatic stimulus. This is in reality the general conclusion arrived at by THOMPSON (8) for *Abies*.

The foregoing résumé of the work already done in this subject is presented in an introduction to a description of a peculiar adaptation in ray tracheid structure noted in second growth wood tissue of *Sequoia washingtoniana* from the Sequoia National Forest in California. The sections were taken from the main trunk of the tree, which shows a phenomenally rapid growth not usually associated with *Sequoia*, attaining in 30 years a diameter of 19 inches at the point of section. Growth was kept up fairly regularly and consistently during the entire period.

The wood was very light in weight and very soft, was very easily cut with a knife, and capable of successful sectioning with no further treatment other than boiling. In texture the wood was harsh and coarse and somewhat inclined to be cross-grained. Sapwood was very prominent, comprising more than 90 per cent of the cross-sectional area. The growth rings were wide spaced, varying from 0.2 to 0.7 inches for a single season's growth. Microscopically the cell structure was large and thin-walled, with a very gradual transition from spring to summer wood.

As has already been stated, ray tracheid structure normally occurs in both of the present members of the genus *Sequoia*. In the mature wood of *S. washingtoniana*, two kinds of ray tracheids are to be found. First, the single, isolated, detached, radially elongated tracheary cell found on the upper and lower margins of primary rays, as is shown in fig. 1. The extent of the radial

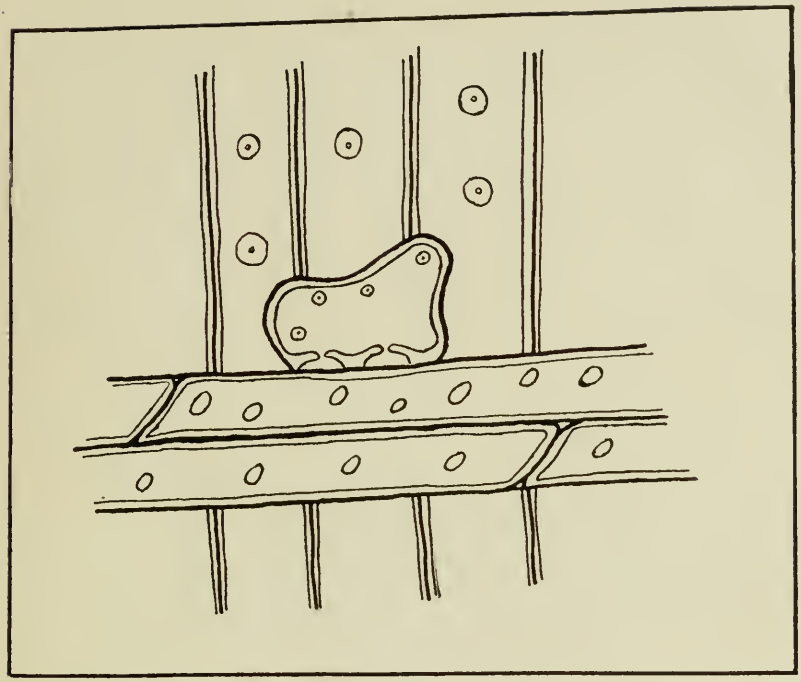


FIG. 1.—*Sequoia washingtoniana* (virgin growth): radial section showing ordinary type of isolated detached ray tracheid.

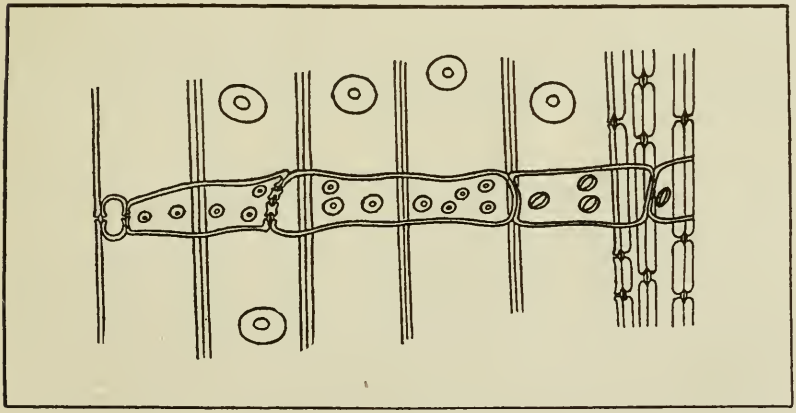


FIG. 2.—Radial section (mature wood) showing ray with two tracheid cells interspersed between ray parenchyma cells; tracheids not marginal, but components of a ray one cell high.

elongation is variable, as is also the height and the depth of the cell, which latter, however, approximates that of the ray with which it is associated. The pitting on the walls is very characteristic, especially in horizontal contact with the parenchyma cell of the ray. Second, the interspersed type of ray tracheid, as shown in fig. 2, where the tracheary cells occur in the midst of the radial prolongation of rays one cell high. They may be found singly, or in groups separated from other cells or groups of cells of similar structure by one or more parenchyma cells. Fig. 2 shows two tracheid cells occurring together with parenchymatous ray cells on either

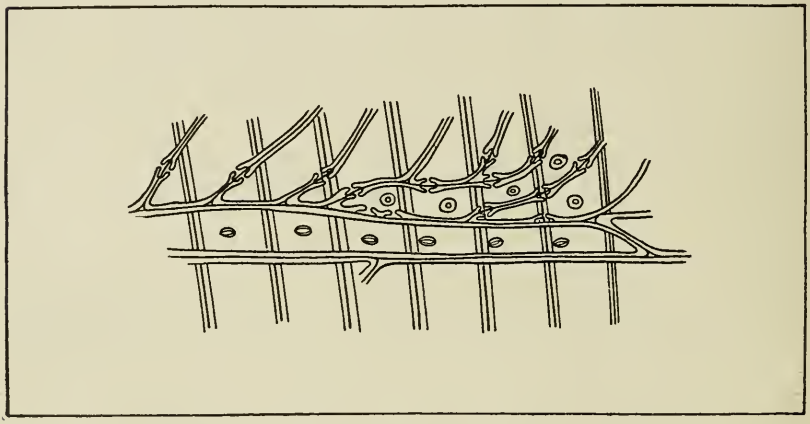


FIG. 3.—Radial section (second growth wood) showing most common form in which vertical wood tracheid is bent and prolonged along the ray to act as ray tracheid; note diversity of pitting in walls of tracheary cell.

side. In these the pitting is very characteristic, especially in the radial end walls. Miss HOLDEN has spoken of such rays as these as "secondary rays."

In contrast to the foregoing, the marginal structures on the rays of the wood of second growth *S. washingtoniana* show great variation. True ray tracheids in accordance with the previous descriptions do not occur. On the margins of the rays, however, there is a peculiar adaptation in the termination of the vertical wood tracheids directly at the ray, with the development of communicating pits in the contiguous walls of the tracheids and the

parenchyma cells of the ray. There is also evolved a radial elongation and projection of the terminating ends of the vertical tracheary elements in direction parallel to and in contact with the parenchymatous cells of the ray, with communicating pits in the intervening walls, as is shown in fig. 3. Inasmuch as true ray tracheids are not to be found, it is believed that these structures are acting as such and possess all the functions attributed to and carried on by ray tracheids.

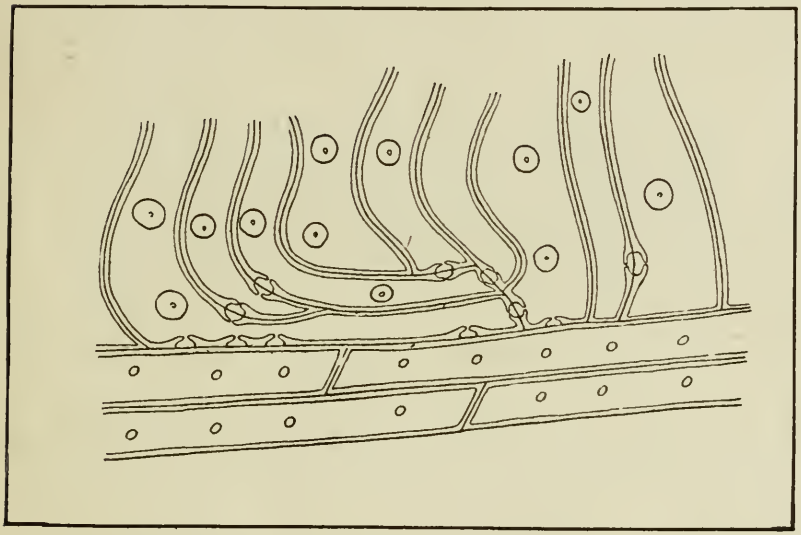


FIG. 4.—Radial section (second growth) showing wood tracheids bent and prolonged to form marginal ray tracheid two cells deep for width of two vertical wood tracheids.

It is also to be noted that in a great many cases the course of the bent-over and prolonged tracheid is imitated by those immediately contiguous with greater or less development. This is especially noticeable in fig. 4, where two neighboring and contiguous vertical tracheary elements are bent over in such a way that a ray tracheid two cells deep is evolved for the distance covered by two vertical tracheids. That these bent-over wood tracheids function as ray tracheids is evident from the double form of pitting to be found in their cell walls.

This procumbent and radial prolongation of vertical tracheary elements is to be found on either the upper or the lower margins of the rays. The direction of the prolongation may be toward the pith or toward the cambium. There seems to be absolutely no constant feature of direction which the bending shall follow, either in orientation of the one with the other or with the pith and the cambium. Along the same ray, as is seen in fig. 5, neighboring tracheids can be found bent in opposite directions and so prolonged that the end walls are in contact.

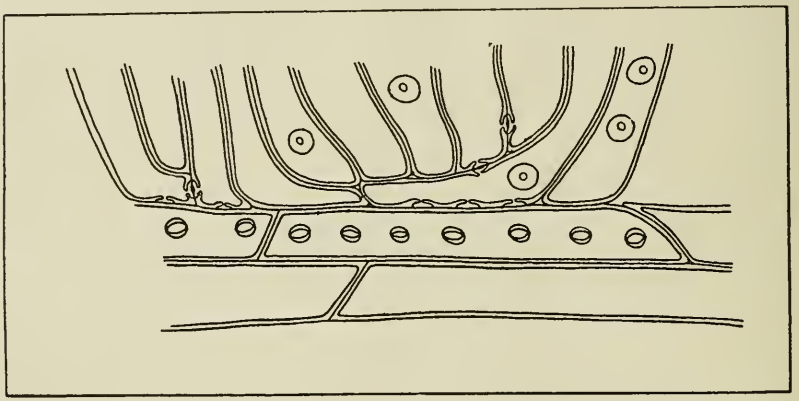


FIG. 5.—Radial section (second growth wood): adjacent wood tracheids bent in different directions along same ray.

In this second growth wood these structures were common to all parts of the stem, pith, medial sections, and cambial layers. Of the three it was perhaps least highly developed in the sections contiguous to the pith, in which there is some development of heartwood. It was found in equal frequency in either the spring or the summer wood, although, as would be expected, it was more clearly defined and capable of better figuring in the large structures of the early wood.

These structures recall and are similar to those described and figured by THOMPSON (7) in the cone axis of *Pinus Strobus*, and by JONES (5) in the mature wood of *Sequoia sempervirens*, in both of which there is ascribed similar function by the respective writers.

No attempt is made in the present article to draw any particular or general conclusions. The whole is submitted as an observation and description of a peculiar and interesting wood structure. It is to be noted, however, that these structures were found and are described in extremely rapidly grown wood tissue, and it is thought that they are special adaptations of the elements for the transference of material between the vertical and horizontal tissues, since there is an entire absence of the usual intermediary tracheary channels of communication. The origin and formation of these latter elements have been fully described and established by THOMPSON (8).

This study was undertaken at the suggestion of Professor S. J. RECORD, of the Yale School of Forestry, who also supplied a considerable portion of the material and much kindly criticism, and to whom the writer wishes to express his thanks and appreciation.

NEW YORK STATE COLLEGE OF FORESTRY
SYRACUSE UNIVERSITY

LITERATURE CITED

1. DEBARY, A., Comparative anatomy of the vegetative organs of phanerogams and ferns.
2. GORDON, MARJORIE, Ray tracheids in *Sequoia sempervirens*. New Phytol. 11:1–7. 1912.
3. GOTHAN, W., Review of JEFFREY's "The genus *Sequoia*," Just's Bot. Jahrb. 31:848. 1903.
4. HOLDEN, RUTH, Ray tracheids in the Coniferales. BOT. GAZ. 55:56–64. 1913.
5. JONES, W. S., Ray tracheids in *Sequoia sempervirens*, and their pathological character. Quart. Jour. Forestry. 1914.
6. PENHALLOW, D. P., The anatomy of the North American Coniferales (p. 88).
7. THOMPSON, W. P., On the origin of ray tracheids in the Coniferae. BOT. GAZ. 50:101–116. 1910.
8. ———, Ray tracheids in *Abies*. BOT. GAZ. 53:331–338. 1912.

PERITHECIA WITH AN INTERASCICULAR PSEUDOPARENCHYMA

F. L. Stevens

(with plate xxx)

Taxonomic import is attached to the presence of paraphyses between the asci in the perithecium or other ascigerous structure. For this reason, as well as on purely morphological grounds, the structure to be here described is of interest.

The collection was made March 31, 1913, at Jayuda, Porto Rico, on the common Maya (*Bromelia pinguin*). Large leaves or portions of leaves were dead and rather thickly set with intensely black bodies, which on microscopic examination were readily revealed as perithecia, bearing abundant asci. Ordinary examination of material boiled in water or in potash solution, then teased apart and crushed, showed no strikingly unusual features about the asci, except that it was difficult to decide whether or not paraphyses were really present. Material was softened in lactophenol for two days, washed, and then imbedded in paraffin through xylol, and sectioned.

From the sections it is clearly apparent that the black, thick, perithecial wall is sharply limited on its inner side, and that the central area, which in most perithecia is merely a cavity or a cavity partially filled with asci and paraphyses, is in this case occupied by a pseudoparenchyma. The perithecial wall cells are dark and thick-walled (figs. 2–3). The interascicular pseudoparenchyma is composed of thin-walled, hyaline cells, small and of quite uniform size. In relatively old perithecia with mature asci the spaces between and above the asci are completely filled with the pseudoparenchyma. In still older perithecia the interascicular pseudoparenchyma is seen to break down, beginning at the ostiole. An example of this is shown in fig. 1. As the ostiolar tissue disorganizes a mycelium penetrates down through it; whether this mycelium belongs to this fungus or to another is not known

(fig. 1). In young perithecia which do not yet show asci, the whole central portion of the perithecium is filled by the pseudoparenchyma (fig. 2). It is of interest to know how the asci develop within this structure, but the material did not afford all the evidence desired. The asci, however, are all basal and arise from any part of the base of the perithecium (figs. 3, 4), and since asci of various ages are seen imbedded in the pseudoparenchyma, it is apparent that they grow out into it; and since there is no evidence of crowding exhibited by the pseudoparenchyma cells near the asci, the asci probably digest the pseudoparenchyma as they grow forward. Indeed the pseudoparenchyma is very tenuous and is probably very easily disposed of. Fig. 4 shows an ascus that has shrunk, leaving a free space between itself and the surrounding pseudoparenchyma. The structure of this perithecium suggests the condition in the perithecium of *Penicillium* and the Plectascineae generally, where the asci are scattered in a pseudoparenchyma. This case is different, in that the asci in this fungus are not scattered but arise basally. The similarity, however, suggests a relationship between the Plectascineae and the Sphaeriales.

In the Erysiphaceae the asci develop *pari passu* with the perithecium, and at certain stages may show asci with parenchyma-like cells between them (cf. fig. 30, pl. 2., HARPER, R. A., Carnegie Inst. Publ. 37, September, 1905). In certain other fungi the young perithecium is solid throughout and pseudoparenchymatous; while later the central cells disorganize and a central cavity results. The asci push up into this cavity. Neither of these conditions presents an exact parallel with that of the fungus under consideration. A simple rational inference is to regard the case as one of delayed dissolution of the pseudoparenchymatous central region of the developing perithecium. The fact that this structure was not clearly seen without good thin sections raises the question whether similar conditions may not exist in other perithecia, and may have been overlooked because the microtome has not been employed.

The mycelium of this fungus is interesting on account of its great variation in shape and size (fig. 5). The ostiole is lined by

a fringe of projecting toothlike or clawlike cells. The perithecia often occur solitary, when they are seen to be clearly sphaeriaceous in character. Often there are two (fig. 6), more rarely three, lying together. Such considered by themselves might be regarded as dothideaceous. The fungus appears to be clearly sphaeriaceous, and owing to the peculiar character of the pseudoparenchyma I propose it as a new genus.

Desmotascus, gen. nov.—Mycelium and perithecium black, sphaeriaceous, ostiolate, short-beaked. Asci with an interascicular pseudoparenchyma, 8-spored. Spores nearly hyaline, 1-celled.

It differs from *Phomatospora* in the character of the interascicular pseudoparenchyma. Name from δεσμώτης, prisoner. The type is the following:

Desmotascus portoricensis, sp. nov.—Mycelium dark, varying in diameter from 4 to 17 μ. Perithecia 119–190 μ wide, 85 μ high, black, roughly spherical, solitary or in groups of two or three, immersed papillate or short-beaked, ostiolate. Asci 8-spored, oblong, obtuse, thickened at apex, 50–85×17 μ. No paraphyses, but the perithecial cavity filled by a pseudoparenchyma. Ascospores oblong, somewhat irregular, 20–31×8.5–10 μ, pale straw-colored.

On *Bromelia pinguin*, Mayaguez, 964 (type); 964–1 type slide.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

EXPLANATION OF PLATE XXX

FIG. 1.—Ostiole with mycelium entering and disorganizing the tissue.

FIG. 2.—Whole interior of perithecium filled with pseudoparenchyma (*h. p.*).

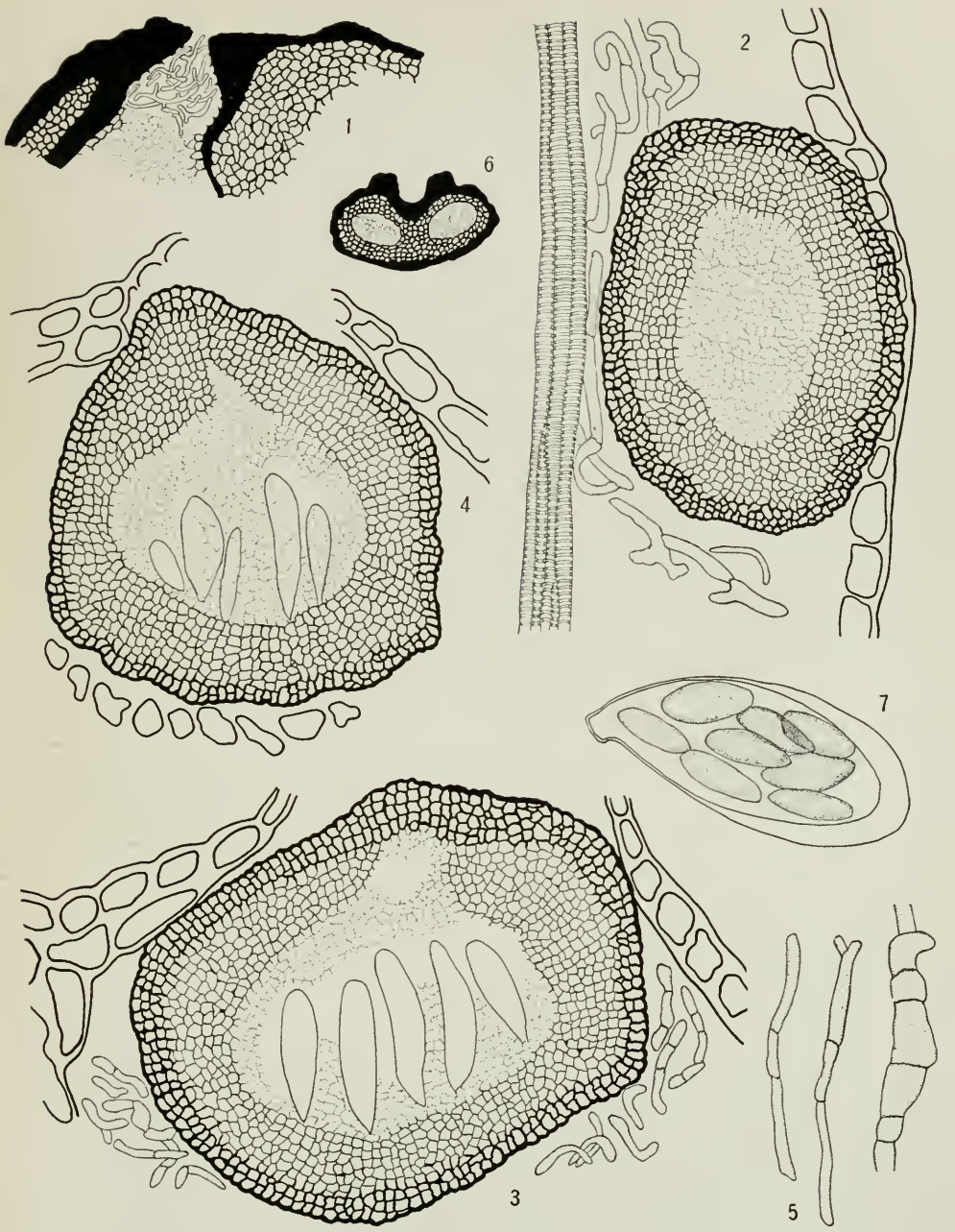
FIG. 3.—Perithecium with asci, showing origin from various parts of base of perithecium.

FIG. 4.—Showing asci shrunk away from interascicular pseudoparenchyma (*h. p.*).

FIG. 5.—Showing variation in size and shape of internal mycelium (*h. p.*).

FIG. 6.—Two perithecia showing beaks (*l. p.*).

FIG. 7.—Ascus and spores (*h. p.*).



STEVENS on PERITHECIA

CURRENT LITERATURE

BOOK REVIEWS

Plant succession

CLEMENTS has brought together in a satisfactory way and in sumptuous form the contributions of all previous workers dealing with the phenomena of succession in vegetation.[1] The work of the various students of succession is conscientiously and sympathetically presented, and with great fulness. So admirably is this work done that it will henceforth be largely unnecessary to refer to original publications, prior to the appearance of this work, in order to get the substantial views of the various authors. The work is thus a compendium of our knowledge and theories bearing on the phenomena of succession. In the field covered by this work, CLEMENTS is himself a major contributor, and in no previous work has he contributed so much new material on the subject as this work includes. No more can be attempted here than to touch a few of the high spots.

As previously, CLEMENTS treats the formation as an organism, with structures and functions like an individual plant. As compared with previous studies by the same author, greater stress is placed on development and less on habitat. To the reviewer this seems a distinct step forward, although many workers, especially in Europe, will continue to emphasize habitat as the controlling factor in classification. The formation is defined as "the climax community of a natural area in which the essential climatic relations are similar or identical." Thus CLEMENTS' formation, as here presented, departs materially from the concept of the Brussels Congress, but agrees essentially with the "climax formation" of the reviewer, and with the still earlier "climatic formation" of SCHIMPER. SCHIMPER, however, probably failed to recognize that his "climatic formation" was really the topmost member of a series of his "edaphic formations." A number of new terms of classical origin are introduced in this volume, as is the wont of the author. Perhaps the most important of these is "sere," a term used to include the entire successional series leading up to the climax. This term is used, rather than its essential equivalent "series," because of its adaptability in combination, as in xerosere (a xerarch series), etc. CLEMENTS' treatment of the term "climax" is in general harmony with the often expressed interpretation of the reviewer; "the climatic formation is the real climax of the successional

[1] CLEMENTS, F. E., Plant succession; an analysis of the development of vegetation. pp. xiii+512. *pls. 436. figs. 51.* Carnegie Institution. Washington. 1916.

development." An apparent climax, short of the true regional climax, is termed a subclimax.

The chapter in which the views of the author and the reviewer clash most sharply is the one on direction of development. CLEMENTS states positively that "succession is inherently and inevitably progressive." The reviewer is as positive in his opinion as ever that succession may be retrogressive as well as progressive, although of course progression is much more abundant and important. What the reviewer would term retrogression is for the most part by CLEMENTS termed denudation, preparatory to the initiation of another successional series. This might pass, if all such denudations or retrogressions were sudden, resulting at once in the development of a habitat initial to a progressive series. In an area that is gradually sinking, there may be a gradual retrogression from a climax mesophytic forest to a hydrophytic association, with no denudation of any sort whatever. In a review of CLEMENTS' work by TANSLEY,[2] it is shown that it would be very difficult to apply to England the idea that succession is always progressive.

Chapters follow on the classification of "seres," the climax formations of North America, past climates and climaxes, and past succession. The chapters on past climates and vegetation will be of great value, because they bring together compactly results from widely scattered sources. The theories and the applications of the author's views to the past seem very tenuous. It is difficult enough to apply ecological principles to the vegetation of the present, and it is very much too soon to work out the characteristics and successions of past floras in any but the most superficial manner.

The work is a notable one, and must be on the working table of every ecologist and plant geographer. It is unfortunate, however, that the author has allowed his splendid classical training and love for Greek and Latin to carry him so far afield. The tendency nowadays is toward increasing emphasis on the vernacular, and it is to be feared that many of the author's best thoughts and most inspiring ideas will remain hidden among words.—HENRY C. COWLES.

Botany of the living plant

BOWER[3] has put into book form his course of lectures on elementary botany given at the University of Glasgow for more than 30 years. He gives a vivid picture of the plant as a living, growing, self-nourishing, self-adapting creature.

Of the 32 chapters, 18 are devoted exclusively to angiosperms, whose complete life activity is exhaustively treated from seed "germination" to seed dispersal. In the one chapter devoted to gymnosperms only the Scots pine is treated. This seems to the reviewer to be very inadequate treatment

[2] TANSLEY, A. G., The development of vegetation. Jour. Ecol. 4:198–204. 1916.

[3] BOWER, F. O., Botany of the living plant. 8vo, pp. x+580. *figs.* 447. Macmillan Co. 1919.

of such a fundamental group. In the two chapters assigned to Pteridophytes, only the Lycopodiales and Filicales are discussed. In the two chapters dealing with Bryophytes, students of this group will be surprised to learn that in Hepaticae the sporophyte, "except in the peculiar group Anthoceroteae, does not carry on photosynthesis." A statement concerning the ventilated structures of the gametophyte of Bryophytes is likely to mislead any but intimate students of the group: "In the sporophyte of vascular plants the typical photosynthetic organ is the leaf blade with its ventilated mesophyll and stomatal control. In the gametophyte of mosses and liverworts a similarly ventilated structure is seen in the leaves of the larger mosses and in the thallus-structure of the Marchantiales. These are, however, parts of the gametophyte, and the ventilated structure is here produced mainly by involution of the outer surface, while in vascular plants it arises from intercellular splitting of the cell walls. The physiological end is the same, in both cases, but the place and means are different. Plainly these are the results of parallel evolution, or homoplasy." Had the actual facts in Marchantiales been stated, the proof of homoplasy would have been absolute. Thallophytes are dealt with in 8 chapters. In the introductory chapter a statement widely applicable to all plant phyla is made: "It must not be assumed that all the organisms grouped under a common designation are necessarily akin to one another. . . . . Lines of descent are *divergent*, and the thallophytes would appear to represent a brush of phyletic lines radiating outwards from some simpler source." The chapter on sex and heredity is presented so clearly, so free from all verbiage, that beginners can easily grasp this subject, usually considered so complex. Likewise, the chapter on alternation of generations, the land habit, and the rise and fall of the gametophyte, is a masterpiece of lucid expression.

This text is the work of a man notable for brilliant research and also for exceptional power as a teacher. It is an example of the results obtained by a long period of first-hand contact with material as well as the presentation of these results to many generations of students.—W. J. G. Land.

Forest products

As the utilization of plant products is a matter of interest to all botanists, a book discussing the use of wood and forest products, other than lumber, should find a place in all botanical libraries. The present volume[4] is attractive in appearance, well illustrated, and carefully organized. Some idea of its scope may be obtained from such chapter headings as the following: Wood pulp and paper, Tanning materials, Veneers, Slack and tight cooperage, Naval stores, Wood distillation, Charcoal, Boxes, Cross ties, Poles and piling, Mine timber, Fuel, Shingles, Maple syrup and sugar, Rubber, Dye woods, Excelsior and cork. Under each topic the character and source of the raw material,

[4] Brown, N. C., Forest products. 8vo. pp. xix+471. *figs. 120.* New York: Wiley & Sons. 1919. $3.75.

the tree species involved, the processes of manufacture, the marketing, the utilization, and values are discussed. Whenever any attempts have been made toward standard specifications and grading of the products, these are given in considerable detail. Statistics of production in the United States or of importation from other lands are arranged in convenient tables, and still more important for the scientist is the bibliography which is appended to each chapter. Costs of raw material, labor, overhead, and marketing are considered, as well as selling prices and total value of production; while a detailed index makes this mass of information available for ready reference.—GEO. D. FULLER.

Economic woods

RECORD'S[5] presentation of the subject of wood structure has already made his book, in its first edition, indispensable in all laboratories where the identification of wood is attempted, on the basis of its structure as revealed through the microscope. The volume has also proved equally useful in classes where the general principles of wood structure are being studied, hence an enlarged second edition will be welcomed by a considerable constituency.

Among the desirable features of the work are good clear illustrations (whose number might be increased to advantage), logical organization, concise statement, convenient tables for reference, and a well arranged, excellent bibliography, which in the present edition is brought down to 1918. The identification key has been revised and improved and appears adequate to the demands likely to be made upon it.

One of the features of the new edition is an appendix devoted to a general description of the woods of the United States and their classification on a structural basis. Tables giving the occurrence of such structures as pits, spiral markings, and tyloses in various genera and species afford convenient means of classification and of easy reference.—GEO. D. FULLER.

NOTES FOR STUDENTS

Influence of a crop on succeeding one.—HARTWELL and his associates[6] have done some very important work on the influence of crop plants on those which follow. Some crops are very injurious to those which follow them, while other successions reveal no injurious action. As is shown by an illustration on the front cover of Bulletin 175, buckwheat is greatly injured when it follows millet, but shows good development when it follows turnips. The method,

---

[5] RECORD, S. J., Identification of the economic woods of the United States. 8vo. pp. 157. *pls. 6. figs. 15.* New York: Wiley & Sons. 1919. $1.75.

[6] HARTWELL, B. L., and DAMON, S. C., The influence of crop plants on those which follow. Bull. 175. Agric. Exp. Sta. R.I. State College. 1918.

HARTWELL, B. L., PEMBER, F. R., and MERKLE, G. E., The influence of crop plants on those which follow. Bull. 176. Agric. Exp. Sta. R.I. State College. 1919.

results, and significance of these experiments can best be presented by quotations from the summaries of the two bulletins:

"The general plan of the field experiment, which is the main subject of this bulletin, is to grow 16 different crops on that number of plats for two seasons prior to growing a different one of the crops over the entire area every third year. No farm manures are used, but fertilizer chemicals are applied on all plats alike, in amounts intended to supply an average of the nutrient needs of the different crops. Information regarding these needs is obtained by soil tests conducted in pots at the greenhouse and in sections of drainpipe sunk in the paths between the field plats.

"Onions occupied the entire area in 1910. If the preceding crops are arranged in the order of increasing yields of onions of the first class, it is seen that 13–17 bushels of onions per acre were produced following cabbages, mangel beets, rutabaga turnips, and buckwheat; 35 and 87 bushels following potatoes and rye; 131–178 bushels following corn, millet, onions, oats, and red clover; 240–314 bushels following squash, timothy, and alsike clover; and 406 and 412 bushels following mixed timothy and redtop, and redtop alone.

"In 1913, after the miscellaneous crops had been grown on their respective plats again for two years, buckwheat was planted on the entire area. Again arranging the crops in accordance with increasing yields, it follows that only 4–10 bushels of buckwheat grain were produced where millet, grasses, corn, and clovers had been growing previously; 13–15 bushels where buckwheat and oats were the preceding crops; 20–23 bushels where the preceding crops had been cabbage, beets, onions, rye, squashes, and potatoes; and 34 bushels following turnips.

"Alsike clover was chosen for the crop next grown on the entire area. The lowest total yields of clover hay for the two years 1916 and 1917 were 2.53–2.60 tons per acre, following the clovers and carrots. The highest yields were 4.16–4.33 tons, following rye and redtop, and two years' failure of squashes. Intermediate yields of 3.31–3.86 tons were secured following the remaining crop plants.

"The divergent effect of crops on those which follow seems not to be attributable, at least principally, to differences in the amount of nutrients removed by the crops grown previously; that is, the smallest yield may not occur after the crop which removed the largest amount of even the most-needed nutrient. The soil acidity was affected differently by the several crops and, generally, the best yields of the onion, a plant which is sensitive to conditions accompanying acidity, followed the crops giving rise to the least acidity. These indications assume added importance because of the observed fact that the effects of the crops on those which follow were much less divergent if the soil acidity was reduced by liming.

"Even if later work should prove that preceding crop effects are not important in connection with a neutralized soil, attention should nevertheless be given by the practical farmer to the very potent influences which have been

observed in the present work, for the reason that so many soils have a greater degree of acidity than existed in these experiments, and it is doubtful if they will ever be limed sufficiently to maintain them in a neutral condition."

This work should be of great interest to the ecologist and the physiologist, as well as to the agriculturist.—WM. CROCKER.

Upper Cretaceous floras.—The eastern gulf region in Tennessee, Alabama, and Georgia, discussed by BERRY[7] with reference to the Upper Cretaceous floras, includes that part of the Atlantic coastal plain bordering on the Gulf of Mexico and lying south and west of the southern Appalachian province and east of the Mississippi River. An excellent map in colors shows the exact geographic location of the different geological formations which contain determinable plant fossils, and it appears that the bulk of the fossil floras belong to the Tuscaloosa formation, with those of the Eutaw and Ripley floras meagerly represented.

The author gives a systematic arrangement of the plants found in the Upper Cretaceous of the Gulf region, with a historical sketch, an account of the lithologic characters of the materials associated with the fossils, and a discussion of the localities with plant remains. Photographs and diagrammatic sections help to elucidate the account arranged according to the different localities where plant fossils have been found. After a thorough analysis of the field observations made separately for the Eutaw, Ripley, and Tuscaloosa formations, the composition, origin, and evolution of these different Upper Cretaceous floras follow. The 151 described species from the Tuscaloosa formation represent 87 genera segregated into 48 families. The pteridophytes are represented meagerly, while the cycad-like plants, abundant in the Lower Cretaceous, are represented by a single species of *Podozamites* and *Cycadinocarpus*. Sixteen species of Coniferales of modern types, as *Pinus*, *Dammara*, *Sequoia*, occur with the curious extinct phylloclad type, *Andcovettia*, etc. The angiosperms constitute the bulk of the Tuscaloosa flora. The author explains the scarcity of the monocotyledons as largely due to the fact that the lack of differentiation of the leaf lamina and petiole precludes the regular shedding of their leaves, which are torn to shreds by the wind, and therefore unrecognizable. The dicotyledons of the Upper Cretaceous are of great interest as to their origin, for they appeared with great suddenness at the close of the Lower Cretaceous in America, Europe, and the Arctic region. The author believes that North America was near their center of radiation, with the facts in accord with their Arctic origin and with successive waves of migration sweeping southward.

Dealing specifically with the Tuscaloosa formation, BERRY emphasizes its delta character with its flora of a lowland coastal type, including a number of distinctly strand types, such as the species of *Murica*, the figs, and several

[7] BERRY, E. W., Upper Cretaceous floras of the eastern gulf region in Tennessee, Mississippi, Alabama, and Georgia. U.S. Geol. Survey, Professional Paper no. 112. pp. 117. *pls. 1–33*. 1919.

Lauraceae, Leguminosae, and Celastraceae. The members of the Myrtales also were probably dwellers in the dry or wet strand. Many of the plants probably demanded a heavy rainfall, with emphasis on the warm temperate rain forest types. Such areas as southern Japan or northern New Zealand offer many points of comparison with the Upper Cretaceous floras of the coastal plain. The climate then was equable within the limits embraced between warm temperate and subtropical. The floras of the Eutaw and Ripley formations are treated similarly by BERRY, who concludes the introductory portion of the monograph with a consideration of correlations and the presentation of a table of the distribution of the three floras minutely analyzed. The work ends with a detailed account of the fossil plants, with the description of several new species illustrated with 33 fine plates of geological scenery and fossil plants.—J. W. HARSHBERGER.

Wood structure and conductivity.—HOLMES[8] has made a quantitative study of the anatomy of ash wood, attention being directed chiefly to the size and proportion of the water-conducting elements in different parts of a shoot. As in the case of the hazel wood previously investigated by this author, year old ash shoots were selected, most of the specimens being typical coppice, stool shoots, long, thick, and unbranched. His results are presented in graphical form, a set of curves being constructed for each shoot.

Curve A gives the variation in area of the wood at selected levels along each shoot. Curve B, representing the absolute conductivity or total volume of transmitted water, is obtained by calculating the total number of vessels in a transverse section at the different levels and the average diameter of the cavities of these vessels. This curve shows a decline from the base to the apex of the shoot. Curve C serves as a measure of the specific (or relative) conductivity for water, or the percentage of wood area occupied by vessel cavities. In general this curve rises and then falls, the upper (younger) part of the stem being a better conductor of water per unit area than that nearer the base. An increase in the proportion of fibers in any part of the shoot, usually at the base where mechanical support is necessary, quite obviously lowers the specific conductivity in that portion of the stem.

In comparing the ash with the hazel wood, the writer finds in both a fall in absolute conductivity and a rise in specific conductivity from the base of the shoot to its apex, but the figures for specific conductivity are much higher in hazel than in ash, due to its greater number of conducting elements per unit area.

In the main, these results agree with those obtained by FARMER[9] for the two kinds of wood in question, in his extensive investigations for determining

[8] HOLMES, M. G., Observations on the anatomy of ash-wood with reference to water-conductivity. Ann. Botany **33**:255–264. *figs.* 7. 1919.

[9] FARMER, J. B., On the quantitative differences in the water-conductivity of the wood in trees and shrubs. Proc. Roy. Soc. **90**: 1918.

specific and absolute conductivity. FARMER calls attention further to the close resemblance between coppice-shoots and saplings of the ash and hazel in respect to their water-conducting systems, and to the difference existing between the coppice-shoots and the normal adult wood of these species.—LADEMA M. LANGDON.

After-ripening and germination of rice.—KONDO[10] has done some very interesting work on the germination of rice seeds. Seeds gathered in the milk stage and put into a germinator immediately show little germination, even after 30 days. Those stored in a condition permitting drying for 15 days, or those stored without drying for 30 days, after-ripen and show a considerable improvement in germination. With after-ripening germination sometimes exceeds 50 per cent. Seeds harvested in the yellow ripe stage show little germination when immediately placed in a germinator, but they improve in germination relatively rapidly with storage, whether the storage conditions permit drying or not, and after 4 months of storage give as good germination as seeds harvested fully ripe. Seeds harvested fully ripe germinate fairly well immediately, but are considerably improved by after-ripening. Seeds harvested dead ripe do not need after-ripening, but are immediately capable of prompt and good germination.

While drying hastens the after-ripening of seeds collected in the milk or yellow ripe stage, those after-ripening without drying finally give quicker and better germination than those after-ripened with drying. The presence of the hulls interferes with after-ripening. A few hours of sun-drying of the fresh seeds favors germination. Diffuse light has no effect on the germination of fully ripened seeds, but it favors the germination of those not fully after-ripened. Germination percentage and energy both rise with progress in the maturity and after-ripening of the seeds. Many grains of rice show abnormal germination. In many of the seeds collected in the milk stage only the radicle grows. In the yellow ripe, fully ripe, and dead ripe grains the abnormality is shown by the growth of the plumule only, often followed later by many secondary roots.

The matter of dormancy and after-ripening of cereal seeds is giving seed testers and other practical workers no little concern, especially in regions where ripening occurs during cool or wet weather.—WM. CROCKER.

Anthocyanin.—The distribution of anthocyanin in varieties of *Coleus hybridus* has been studied by KÜSTER,[11] who classifies the patterns in two groups: (1) sectional, mottled, and pulverulent; (2) areas with curved boundaries and circular flecks. These groups of patterns are traced to different origins. Patterns of the first group are traced to qualitatively

[10] KONDO, MONTARO, Über Nachreife und Keimung veschieden reifer Reiskorner. Ber. Ohara Inst. Landw. Forsch. 1:361–387. 1919.

[11] KÜSTER, ERNST, Die Verteilung des Anthocyans bei Coleusspielarten. Flora 110:1–33. 1917.

unequal cell divisions by which unlike daughter cells arise, one of which possesses ability to produce anthocyan, the other lacking it. In all future divisions of the anthocyanin-producing mutated cells, the daughter cells also inherit the power to produce the color. The contiguous mass of colored cells in a sectional, mottled, or pulverulent pattern is considered the product of a single mutant cell. If the mutation occurs at a very early stage in the life of the plant, sectorial coloration is likely to result. If somewhat later, after the main organs have been laid down, the mottled pattern results. When the cell mutation occurs very late, so that only a few daughter cells are formed by each mutant, the pattern is pulverulent.

Patterns of the second group, rounded areas, and flecks of anthocyanin occur more rarely than those of the first group. Comparison of these patterns with the first indicates that they do not arise by cell mutation. Using seed crystals as an illustration, he suggests the possibility that at certain points anthocyanin-producing "seed colloids" of unknown composition arise, and that around these central points aggregation continues, molecules or molecular groups coming from surrounding cells, which are thus left colorless. This hypothetical colloidal substance would have some direct or indirect relation to the production of anthocyanin, either as a source of building material, or as a catalytic agent.—Chas. A. Shull.

Quantitative nature of sex.—Schaffner[12] has published some significant observations on sex intermediates. The white mulberry shows about 40 per cent pure staminate plants, 40 per cent carpellate, and 20 per cent intermediate in all gradations. Among the last, the most interesting example consists of a pure staminate tree with a single, almost pure carpellate branch, showing "that a sex reversal can and sometimes does take place in an old tissue whose cells are removed by thousands of vegetative divisions from the original zygote. It assures us that sex control is only a matter of finding out how to change the prevailing physiological state." The peach leaf willow showed only 9 per cent intermediates. These were primarily staminate, but had many catkins which were staminate only at the base and became carpellate at the end. "But on the transition zone, between the staminate and carpellate parts, the axis seemed to be neutral in regard to sex, and here bisporangiate flowers were frequently present." Also, in this neutral zone abnormal flowers were very frequent, structures developing which were partly staminate and partly carpellate. These observations serve to support the conclusions published by the author in 1910 to the effect that "sexuality is a condition and not a character" (factor). Observations of much the same nature have recently been published by Stout.[13]—Merle C. Coulter.

---

[12] Schaffner, John H., The nature of the dioecious condition in *Morus alba* and *Salix amygdaloides*. Ohio Jour. Sci. 19:409–416. 1919.

[13] Stout, A. B., Intersexes in *Plantago lanceolata*. Bot. Gaz. 68:109–133. *pls. 12, 13*. 1919.

Absorption limits.—The conditions existing in roots while in equilibrium concentration (equal absorption and leaching of ions) with the surrounding solution have been studied by HARVEY and TRUE[14] in sweet corn, squash, peanut, and soy bean. The value of the equilibrium concentration was found to be specific for each plant. Thus for sweet corn it was $12-15\ N \times 10^{-6}$, while for squash it was $35-40\ N \times 10^{-6}$, and for the peanut $50\ N \times 10^{-6}$. It was independent, however, of the kind of electrolyte used, or of the original concentration or volume used, provided the original concentration was non-toxic, and that the volume contained less salt than the plant requires for full growth, so that minimum limits for absorption would be reached. The electrolyte content of the solution after equilibrium has been reached is determined partly by volatile ions (CO_2) which are quantitatively equal for all plants grown under equal conditions if equilibrium with the atmosphere has been established; but it is mainly determined by the rate at which ion-producing compounds of the cell break down, and the rate of reabsorption of these ions. The behavior of roots at minimum concentrations seems to substantiate STILES' view that there are concentration limits below which the root cannot absorb enough salts, merely because the nutrient solution is too dilute.—C. A. SHULL.

Embryogeny in angiosperms.—SOUÈGES,[15] in continuing his studies of embryogeny in angiosperms, has emphasized his claim that the laws which govern development are the same in monocotyledons and dicotyledons. The investigations cited compare typical representatives of monocotyledons and dicotyledons (*Anthericum* and *Polygonum*). According to this investigator the only difference in the embryogeny of the two groups is that in dicotyledons the laws are applicable at the first division of the egg; while in monocotyledons these are not applicable until the second division, the apical cell of the 2-celled embryo being the equivalent of the egg-cell in dicotyledons. It is claimed that the variable behavior of the basal cell of the proembryo of monocotyledons accounts for the differences that have been observed. This thesis is illustrated in detail in the development of the different regions of the embryo.

It is becoming increasingly evident that embryogeny in angiosperms is not represented by two sharply contrasted methods, and these detailed results of SOUÈGES confirm other work dealing only with the cotyledon situation. —J. M. C.

---

[14] HARVEY, R. B., and TRUE, R. H., Root absorption from solutions at minimum concentrations. Amer. Jour. Bot. 5:516–521. 1918.

[15] SOUÈGES, R., Embryogénie des Liliacées. Développement de l'embryon chez l' *Anthericum ramosum*. Compt. Rend. pp. 4. July 1918.

———, Embryogénie des Polygonacees. Développement de l'embryon chez le *Polygonum Persicaria*. Compt. Rend. pp. 3. April 1919.

Some oriental plants.—ROCK[16] has published an account of the endemic arborescent legumes of the Hawaiian Islands. The Islands are poor in arborescent legumes; in fact the whole family is sparingly represented as compared with such families as Rubiaceae, Rutaceae, and Lobeliaceae. The genera discussed, which include endemic arborescent forms, are *Acacia* (3 spp.), *Mezoneurum* (1 sp.), *Sophora* (3 spp.), and *Erythrina* (1 sp.). The paper includes numerous and remarkably fine photographs of herbarium specimens and of the plants in the field.

The same author[17] has presented the remarkable endemic genus *Kokia*, a relative of the cotton, in which he recognizes 3 species and a new variety. It is an interesting fact that the Malvaceae have contributed 2 endemic genera to the Hawaiian Islands (*Kokia* and *Hibiscadelphus*).

KOIDZUMI,[18] in continuation of his studies of the flora of Eastern Asia, has described 13 new species of *Pyrus* from Japan.—J. M. C.

Flora of District of Columbia.—HITCHCOCK and STANDLEY[19] have published a manual of the vascular flora of the District of Columbia and vicinity, to replace L. F. WARD'S "Guide to the flora of Washington and vicinity," published in 1881. A general description of this region shows the great diversity of conditions, resulting in an unusually interesting flora. The summary shows 1630 species of vascular plants, representing 646 genera. Of this number, 287 have been introduced, chiefly from Europe. The contribution is a series of analytical keys, so that when the name of a species is reached it has already been characterized. It is a good model for manuals intended only for the recognition of species.—J. M. C.

New tropical American plants.—In a third paper on tropical American plants, STANDLEY[20] has described 76 new species. A synopsis of the Central American species of *Erythrina* recognizes 16 species, 4 of which are new. The Mexican Mimosaceae are represented by 15 new species, 8 of which belong to *Acacia*. A synopsis of the species of *Leiphaimos* from Panama is given, 8 being recognized, 6 of which are new. *Hoffmannia* in Mexico and Central America is credited with 9 new species. The Rubiaceae of North America are increased by 7 new species in various genera. Miscellaneous species belonging to several families are also included.—J. M. C.

---

[16] ROCK, J. F., The arborescent indigenous legumes of Hawaii. Board of Agric. and Forestry, Bull. 5. pp. 53. *pls. 18.* 1919.

[17] ROCK, J. F., The Hawaiian genus *Kokia*. *Idem.* Bull. 6. pp. 22. *pls. 7.* 1919.

[18] KOIDZUMI, GENITI, Contributiones ad floram Asiae Orientalis. Bot. Mag. Tokyo **33**:123–129. 1919.

[19] HITCHCOCK, A. S., and STANDLEY, P. C., Flora of the District of Columbia and vicinity. Contrib. U.S. Nat. Herb. **21**:1–329. *pls. 42.* 1919.

[20] STANDLEY, P. C., Studies of tropical American phanerogams. Contrib. U.S. Nat. Herb. **20**:173–220. 1919.

New African plants.—The continuous appearance of publications dealing with new plants from Africa is an impressive illustration of the fact that it is still largely an unknown continent botanically. MOORE,[21] in continuation of his "Alabastra diversa," has published 28 new species of African plants, belonging to the following families: Ericaceae (2), Asclepiadaceae (2), Scrophulariaceae (13, 7 belonging to *Buchnera*), Gesneraceae (1), Acanthaceae (1), Verbenaceae (5), Loranthaceae (1), Euphorbiaceae (2).—J. M. C.

Tropical American plants.—BLAKE[22] has revised the American species of *Homalium* (Flacourtiaceae), a genus valued for its timber trees and one that has been puzzling to taxonomists. He recognizes 19 species, 11 of which are described as new. In the second paper cited, 13 new species are described, chiefly shrubs or trees collected in Bahia, Brazil, and Colombia in connection with a general survey of the timber resources.—J. M. C.

New names.—MACBRIDE[23] has given a good illustration of the extensive changes of names necessary to conform to the international rules of botanical nomenclature. In reviewing portions of the Leguminosae, he has published 97 new combinations and 9 new names. In the second paper cited, which deals with miscellaneous families, 28 new combinations are published.—J. M. C.

New species of Vernonia.—In studying the tribe Vernonieae for presentation in *North American Flora*, GLEASON[24] has discovered 6 new species and 9 new varieties. He has also segregated *V. lepidota* Griseb. as a new genus (*Ekmania*).—J. M. C.

---

[21] MOORE, SPENCER L., Alabastra diversa. XXXI. 1. Miscellanea Africana. Jour. Botany 57:212–219, 244–251. 1919.

[22] BLAKE, S. F., The genus *Homalium* in America. Contrib. U.S. Nat. Herb. 20:221–225. 1919.

———, New South American spermatophytes collected by H. M. CURRAN. *Idem.* 237–245. 1919.

[23] MACBRIDE, J. F., Notes on certain Leguminosae. Contrib. Gray Herb. N.S. no. 59. 1–27. 1919.

———, Reclassified or new spermatophytes. *Idem.* 28–39. 1919.

[24] GLEASON, H. A., Taxonomic studies in *Vernonia* and related genera. Bull. Torr. Bot. Club 46:235–252. 1919.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italics*.

A

Aaronsohn, Aaron, sketch of 388
Absorption limits 486
Acacia 487
African plants 488
After-ripening and germination of rice 484
Alaria 151
Allen, E. R., work of 71, 72
Amaranthus, chemical constituents of 313
American plants, new tropical 487, 488
Amylase, secretion of by roots 460
Angiosperms, companion cells of 451; embryogeny in 486
Anthocyanin 484
Arber, E. A. N., work of 152
Arthur, J. C. 147
Aspergillus, zinc and growth of 391

B

Bambekea 232
Barley, relation of nutrient solution to cell sap 297
Basidiomycetes, life history and sexuality of 67
Bassler, H. 73
Belyea, H. C. 467
Bensaude, Mathilde, work of 67
Berry, E. W., work of 482
Black, O. F., work of 69
Blake, S. F., work of 488
Bog water, colloidal properties of 367
Bonazzi, A. 194
Bower, F. O., "Botany of the living plant" 478
Britton, N. L., work of 391
Brown, N. C., "Forest products" 479
Bryophytes, multiple eggs in 392
Burger, O. F. 134
Butters, F. K., work of 149

C

Cactaceae 391; perennating fruit of 151
Cantheliophorales 84
Cantheliophorus 73; cultriformis 98; **ensifer** 99; **grandis** 98; linearifolius 97; mirabilis 101; **novaculatus** 99; **pugiatus** 101; riparius 101; **robustus** 100; **sicatus** 100; **subulatus** 98; waldenbergensis 101
Carbohydrates of Amaranthus 329
Cell sap, relation of nutrient solution to 297
Clements, F. E., "Plant succession" 477
Coker, W. C., work of 72
Colletotrichum 244
Colloidal properties of bog water 367
Colorado, phytogeography of 153
Colorimeter and indicator method 232
Companion cells of Gnetum and angiosperms 451
Conductivity and wood structure 483
Congo, flora of 232
Coniothyrium 241
Contributors: Arthur, J. C. 147; Bassler, H. 73; Belyea H. C. 467; Bonazzi, A. 194; Burger, O. F. 134; Cook, M. T. 310; Coulter, J. M. 65, 72, 151, 152, 232, 311, 312, 388, 391, 486, 487, 488; Coulter, M. C. 63, 68, 70, 485; Cowles, H. C. 477; Cribbs, J. E. 262; Crocker, W. 72, 391, 479, 484; Dalby, Nora 54, 222; Dupler, A. W. 345; Fuller, G. D. 149, 479; Harshberger, J. W. 482; Hendricks, H. V. 425; Hill, J. B. 226; Hoagland, D. R. 297; Kempton, F. E. 233; Knudson, L. 460; Land, W. J. G. 392, 478; Langdon, LaDema M. 483; Levine, M. 67; Miller, W. L. 208; Mogensen, A. 393; Osterhout, W. J. V. 60; Ramaley, F. 380; Rigg, G. B. 367; Rose, D. H. 66; Schertz, F. M. 441; Shull, C. A. 70, 71, 232, 308, 310, 312, 392, 485, 486; Smith, R. S. 460; Stevens, F. L. 54, 222, 307, 474; Stout, A. B. 109; Thompson, T. G. 367; Thompson, W. P. 451; True, R. H. 390; Vestal, A. G. 153; Walker, Leva B. 1; Waterman, W. G. 22; Weatherwax, P. 305;

Weaver, J. E. 393; Weston, W. H. 287; Willaman, J. J. 69, 71, 232, 392; Woo, M. L. 313
Cook, M. T. 310; "Applied economic botany" 307
Corn-pollinator 63
Coulter, J. M. 65, 72, 151, 152, 232, 311, 312, 388, 391, 486, 487, 488
Coulter, M. C. 63, 68, 70, 485
Cowles, H. C. 477
Cretaceous floras 481
Cribbs, J. E. 262
Crocker, W. 72, 308, 310, 391, 479, 484
Crop, influence on succeeding one 480
Cunninghamella, sexuality in 134
Cyathea, parasite of 222
Cycas media, polyxylic stem of 208

D

Dalby, Nora 54, 222
Damon, S. C., work of 480
Davisson, B. S., work of 72
Desmotascus portoricensis 476
Dictyuchus, zoospore emergence in 287
District of Columbia, flora of 487
Dodge, C. W., work of 232, 392
Dormancy, physiology of 307
Duggar, B. M., work of 232
Dupler, A. W. 345

E

East, E. M., work of 71
Ekmania 488
Embryo development in Scrophularia 441
Embryogeny of angiosperms 486
Epicoccum 249
Erythrina 487

F

Farmer, J. B., work of 483
Fernald, M. L., work of 149
Florin, R., work of 392
Flower, development in Scrophularia 441
Forest products 479
Free, E. E., work of 70
Fuller, G. D. 149, 479
Fungi, tyrosinase of 392

G

Germination of rice and after-ripening 484
Gleason, H. A., work of 488
Gloeosporium 244
Gnetum, companion cells of 451
Gold, absorption of 392
Grape growing, manual of American 390
Griffiths, D., work of 312
Griggsia **cyathea** 224

H

Harrington, G. T., work of 308, 310
Harshberger, J. W. 482
Hartwell, B. L., work of 480
Harvey, R. B., work of 486
Hedrick, U. P., "Manual of American grape growing" 390
Hendricks, H. V. 425
Hibiscadelphus 487
Hill, J. B. 226
Hitchcock, A. S., work of 487
Hoagland, D. R. 297
Hoffmannia 487
Hoita 65
Holmes, M. G., work of 483
Homalium 488
Huxley, L., "Life and letters of Sir Joseph Dalton Hooker" 65
Hybrid vigor 150
Hydnaceae of North Carolina 72

I

Infection as related to humidity and temperature 66
Intersexes in Plantago lanceolata 109

J

Java, Zingiberaceae of 152
Johnson, D. S., work of 151
Jones, D. F., work of 150
Jones, W. N., work of 68

K

Kelly, J. W., work of 69
Kempton, F. E. 233
Knudson, L. 460
Koidzumi, G., work of 487
Kokia 487
Kondo, M., work of 484
Küster, E., work of 484

L

Land, W. J. G. 392, 478
Langdon, LaDema M. 483
Lauritzen, J. T., work of 66
Lepidophyte, sporangiophoric 73
Leiphaimos 487
Levine, M. 67
Lycopodium reflexum, anatomy of 226

M

Macbride, J. F., work of 488
Macrophoma 238
Martin, J. N., "Botany for agricultural students" 308

Merkle, G. E., work of 480
Mezoneurum 487
Miller, W. L. 208
Mogensen, A. 393
Molisch, H., work of 72
Moore, S. L., work of 488

N

New names 488
Nitrification 194
Nitrite ferment 194
Nitrogen, compounds of Amaranthus 318; fixation 71
Nomenclature, errors in double 147
North American flora 65
North Carolina, Hydnaceae of 72

O

Opuntia 312
Orbexilum 65
Oriental plants 487
Osterhout, W. J. V. 60
Oxalates, distribution of dissolved 72

P

Paraffin solvents 305
Park, J. B., work of 71
Patellina 247
Pediomelum 65
Pember, F. R., work of 480
Perithecia with interascicular pseudoparenchyma 474
Permeability 70, 232
Pestalozzia 245
Philippine plant diseases 310
Phoma 236
Photometry 71
Photosynthesis, apparatus for study of 60
Phyllachora, andropogonis 54; **banisteriae** 54; **bourreriae** 54; **canafistulae** 55; **drypeticola** 55; engleri 55; **gnipae** 55; graminis 56; **heterotrichae** 56; lathyri 56; mayepeae 56; **metastelmae** 57; **nectandrae** 57; **ocoteicola** 57; rourea 58; securidacae 58; simplex 58; tragiae 58
Phytogeography of Colorado 153
Pinus Banksiana, distribution of 149
Plantago lanceolata, intersexes in 109
Pluteus admirabilis, development of 1
Porto Rico, Phyllachoras from 54
Practical botany 307
Psoralidium 65
Psorobatus 65
Psorodendron 65
Psorothamnus 65
Pycnidium, origin and development of 233
Pynaertiodendron 232
Pyrus 487

R

Ramaley, F. 380
Record, S. J., "Economic woods" 480
Reinking, O. A., work of 310
Renner, O., work of 72
Respiration, apparatus for study of 60
Reviews: Bower's "Botany of the living plant" 478; Brown's "Forest products" 479; Clements' "Plant succession" 477; Cook's "Applied economic botany" 307; Hedrick's "Manual of American grape growing" 390; Huxley's "Life and letters of Sir Joseph Hooker" 65; Martin's "Botany for agricultural students" 308; Record's "Economic woods" 480
Rhytidomene 65
Ridgway, C. S., work of 71
Rigg, G. B. 367
Rock, J. F., work of 487
Root nodules 311
Rose, D. H. 66
Rose, J. N., work of 391
Rosendahl, C. O., work of 149
Root systems, development of 22

S

Sacramento plains, vegetation of 380
Schaffner, J. H., work of 485
Schertz, F. M. 411
Scrophularia, development of flower and embryo 441
Self-sterility 70
Septoria 242
Sequoia, ray tracheid structures 467
Sex, new conception of 68; quantitative nature of 485
Sexuality in Cunninghamella 134
Shull, C. A. 70, 71, 232, 308, 310, 312, 392, 485, 486
Shull, G. H., work of 150
Smith, R. S. 460
Soil fertility 312
Sophora 487
Souèges, R., work of 486
Sphaeronaema 239
Sphaeronaemella 243
Sphaeropsis 240
Spinach, mineral absorption in 69
Spratt, Ethel R., work of 311
Staminate strobilus of Taxus canadensis 345
Standley, P. C., work of 487
Steinberg, R. A., work of 391
Stevens, F. L. 54, 222, 307, 474

Stone, H., work of 310
Stout, A. B. 109; work of 485
Succession 477
Suspensor of Trapa 312

T

Tacinga 391
Tansley, A. G., work of 478
Taxus canadensis, staminate strobilus of 345
Thoday, D., work of 72
Thompson, T. G. 367
Thompson, W. P. 451
Thuja occidentalis, distribution of 149
Tilia americana, ecology of 262
Timber curing 310
Tison, M. A., work of 312
Torsion studies in twining plants 425
Transpiration, in Tilia 262; of coniferous and broad-leaved trees 393
Trapa, suspensor of 312
True, R. H. 390; work of 69, 486
Tubaria furfuracea, development of 1
Turgor and osmotic pressure 72
Twining plants, torsion studies in 425
Tyrosinase of fungi 392

V

Valeton, Th., work of 152
Van Alstine, E., work of 312
Vernonia, new species of 488
Vestal, A. G. 153
Volutella 248

W

Walker, Leva B. 1
Waterman, W. G. 22
Water movements in plants 72
Weatherwax, P. 305
Weaver, J. E. 393
Weston, W. H. 287
Wildeman, E., work of 232
Willaman, J. J. 69, 71, 232, 392
Williams, Maud, work of 232
Williamsonia, cones of 152
Wood structure and conductivity 483
Woo, M. L. 313

Y

Yendo, K., work of 151

Z

Zingiberaceae of Java 152
Zoospores, in Dictyuchus 287

NEW BOOKS

A Manual of Style (Revised Edition). By the STAFF OF THE UNIVERSITY OF CHICAGO PRESS. $1.50, postpaid $1.65. An invaluable handbook for writers, editors, teachers, librarians, ministers, lawyers, printers, publishers, proofreaders—for all who need guidance on matters of propriety in style.

The Religions of the World (Revised Edition). By GEORGE A. BARTON, Bryn Mawr College. $2.00, postpaid $2.15. The author has added two new chapters. All great religions considered. Combines the virtues of popular presentation and scholarly accuracy. Presents in an interesting way a large number of facts little known to the general reader.

Current Economic Problems (Revised Edition). Edited by WALTON H. HAMILTON, Professor of Economic Institutions, Amherst College. $3.50, postpaid $3.75. A selection of readings from the widest range of sources. The revision presents new material made available as a result of the war, and the new point of view brought about by the war in many lines of thinking on economic subjects.

Problems of Fertilization. By FRANK R. LILLIE, University of Chicago. $1.75, postpaid $1.90. As the problem of fertilization deals with the union of male and female sex elements, which forms the beginning of each individual's life-history, it has important bearings upon the problems of heredity. It also has close relations to several other fundamental biological problems.

The Revelation of John. Is the Book of Revelation a Mystery to You? By SHIRLEY J. CASE, Professor of Early Church History and New Testament, the University of Chicago. $2.00, postpaid $2.15. This is a popular presentation of the subject and not a technical commentary. The author tells why and when the book was written.

Outlines of Chinese Art. By JOHN CALVIN FERGUSON. $3.00, postpaid $3.15. This volume embraces six lectures delivered at the Art Institute, Chicago. The author presents in a most interesting way a comprehensive survey of Chinese art. Profusely illustrated.

How the Bible Grew. By FRANK G. LEWIS. Crozer Theological Seminary. $1.50, postpaid $1.65. This is the first single work to record the growth of the Bible from its beginning up to the present time. It answers many questions about the Bible.

The Spread of Christianity in the Modern World. By EDWARD CALDWELL MOORE, Harvard University. $2.00, postpaid $2.15. The book gives a survey of the expansion of Christianity since the beginning of the modern era, about 1757, and depicts the movement against the background of general history.

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - - - ILLINOIS

LOOK

for the five alarm clocks that wake up sleepy typewriting. You will find them in the five red keys of the

Self-Starting Remington Typewriter

This machine puts more "go" into your correspondence and insures an increased typing output in the same typing time. Let us send our salesman to tell you more about it.

Remington Typewriter Company
Incorporated
374 Broadway, New York

FINE INKS AND ADHESIVES
For those who KNOW

Higgins'

- Drawing Inks
- Eternal Writing Ink
- Engrossing Ink
- Taurine Mucilage
- Photo Mounter Paste
- Drawing Board Paste
- Liquid Paste
- Office Paste
- Vegetable Glue, Etc.

Are the Finest and Best Inks and Adhesives

Emancipate yourself from the use of corrosive and ill-smelling inks and adhesives and adopt the **Higgins Inks and Adhesives.** They will be a revelation to you, they are so sweet, clean, well put up, and withal so efficient.

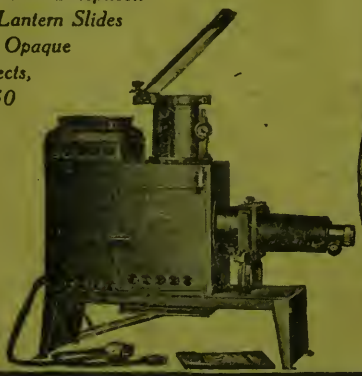
At Dealers Generally

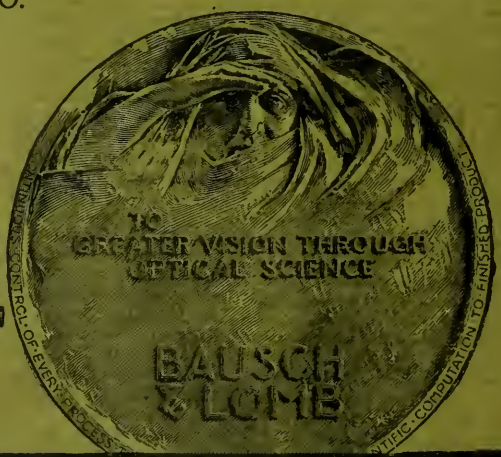
CHAS. M. HIGGINS & CO., Mfrs.
Branches: Chicago, London
271 Ninth Street **Brooklyn, N.Y.**

BAUSCH & LOMB BALOPTICONS

Write for Catalogue
BAUSCH & LOMB OPTICAL CO.
554 St. Paul Street, Rochester, N.Y.

Combined Balopticon for Lantern Slides and Opaque Objects, $150





Bausch & Lomb

New York Botanical Garden Library
3 5185 00259 2440